Bioelectricity Buzz

Recent Bioelectricity-Related Articles Selected by Ann M. Rajnicek, Media Editor of *Bioelectricity*.

A brand-new year for *Bioelectricity* that's buzzing with scientific promise! This year's first instalment covers diverse model systems ranging from plants and hydra to human brain and blood cells. It also reveals how electroacupuncture can impact immune function and how a DNA-based sensor can detect wound infection at a very early stage. A great start for another potentially impactful year for *Bioelectricity* research.

Erythro-exciting: Membrane potential regulates extracellular potentials in red blood cells.

Here is something to make you rethink the classical electrophysiology dogma you learned in school. Hughes et al. use an erythrocyte model to argue that the standard models of cellular electrophysiology should be considered not as independent conceptual units, but as a collective, interdependent, dynamic 'electrome' that incorporates multiple electrical phenomena both at the membrane and in the near vicinity of the membrane. In particular, how the membrane potential impacts extracellular surface potentials.

Hughes MP, Kruchek EJ, Beale AD, Kitcatt SJ, Qureshi S, Trott ZP, Charbonnel O, Agbaje PA, Henslee EA, Dorey RA, Lewis R, Labeed FH. V_m-related extracellular potentials observed in red blood cells. Sci Rep. (2021) 11:19446. doi: 10.1038/s41598-021-98102-9.

In the electrophysiology family, the kid who grabs all the attention is membrane potential (V_m). The famous Goldman-Hodgkin-Katz (GHK) equation describes how V_m is constructed, and this classic equation has set the stage for decades of important discoveries. Although other family members, such as the zeta potential (ζ) and the surface potential (Ψ) play important roles in cell and tissue function, they generally live in V_m's shadow!

That V_m is born from a transmembrane ion gradient is indisputable but classical models, including the GHK equation assume that ion concentrations are uniform within the cytoplasm and in the extracellular space. Furthermore, they assume that V_m is confined to the membrane layer itself and that it does not extend into, or influence significantly, the neighbouring intracellular and extracellular environments.

Hughes and colleagues propose that the standard electrical membrane model is too limiting. They used red blood cells (erythrocytes) to assess quantitatively the interrelationship between V_m and the electrical properties of regions adjacent to the cell membrane.

Within the electrical double layer very near the membrane surface the distribution of ions is determined by co-existing attractive and repulsive electrostatic forces and thermal agitation, leading to the Gouy-Chapman model, which considers the electrical potential as a function of the distance from the cell surface. The electrical potential (ζ potential) measured at ~1 nm from the surface is influenced by double layer thickness and cell surface chemistry (predominantly influenced by charged sialic acid residues). Ion transport underpins the origin and maintenance of V_m so it follows that ion transport will affect ion concentration at the cell surface relative to bulk. Although the GHK equation for V_m includes permeability coefficients to account for this, there is no reciprocal consideration for how the V_m impacts extracellular ion concentration.

Therefore, Hughes and colleagues tested the hypothesis that modifying either V_m or ζ independently would impact the other. They found that ζ influenced erythrocyte V_m but also that altering V_m changed ζ by as much as 37% of V_m, which was attributed to capacitive coupling between V_m and the electrical double layer, rather than from the action of molecular transporters. Furthermore, these properties change dynamically, with V_m exhibiting circadian rhythm-like fluctuations.

Together, the data suggest that the family of electrical properties including ζ, V_m, membrane conductance, membrane capacitance and cytoplasm conductivity comprise a dynamic interconnected cellular electrome (an inseparable family unit) and that an electric field due to V_m consequently extends beyond the membrane.

Brain drain: The unexpected energetic burden of synaptic vesicles.

During the Covid pandemic some of us (ahem) have gained unwanted weight, with a consequent New Year's Resolution to shed those pesky pounds. So, it is heartening to know that even when it's resting the brain burns calories. Pulido et al. reveal synaptic vesicles as a hidden source of substantial resting brain energy expenditure.

> *Camila Pulido, Timothy A. Ryan.* Synaptic vesicle pools are a major hidden resting metabolic burden of nerve terminals. *Sci Adv. 7, eabi9027 (2021). https://www.science.org/doi/10.1126/sciadv.abi9027*.

The brain is clearly a vital organ that must operate constantly and optimally but this comes at a substantial energetic cost. Although the brain represents only ~2 to 2.5% of body mass it consumes about 20% of the body's fuel intake. Electrical activity accounts for some of this expenditure but resting metabolic rates are high too. Pulido et al. hypothesized that resting metabolism could impact availability of ATP (adenosine 5'-triphosphate) in discrete regions as a direct consequence of the need to balance activity-driven demand for ATP with the baseline metabolic needs in those areas. Nerve terminals do not store ATP, so they need to synthesize it locally to meet demand. The group's previous work showed that fuel deprivation halted synaptic vesicle recycling within minutes, pointing to nerve terminals as a site of metabolic vulnerability. Pulido and colleagues have now

expanded this observation by characterising basal metabolic rates at nerve terminals and identifying ion and neurotransmitter transporters as components of the functional mechanism.

The kinetics of presynaptic ATP were assessed in dissociated hippocampal neurons using a quantitative genetically encoded optical reporter. The Na^+/K^+-ATPase pump restores ion gradients, a major metabolic drain in the active brain, and it compensates for leak currents across the plasma membrane in the absence of action potential firing. Consequently, its role in metabolic load was determined during fuel deprivation and in the presence of ouabain, a potent Na^+/K^+-ATPase inhibitor. The authors concluded that the Na^+/K^+-ATPase pump at nerve terminals is not a significant contributor to resting metabolic load, so they queried the role of vesicle resident ATPases (V-ATPases). Blocking V-ATPase activity with bafilomycin under the same conditions used for the ouabain experiments revealed that basal ATP consumption was reduced, such that V-ATPase accounted for nearly half of resting ATP expenditure.

Electrogenic V-ATPase pumps consume ATP in relation to a proton gradient, so a luminal pH reporter was used in nerve terminals to demonstrate that H^+ flux from the synaptic vesical lumen exists, even when there is no vesical cycling or exocytosis. These observations suggest that V-ATPase contributes significantly to sustained ATP consumption. Synaptic vesicle filling uses vesicular transporter molecules driven by the V-ATPase and in some cases the transporters exchange protons, so the group studied the vesicular glutamate transporter (vGlut) family using knockdown of vGlut1 expression in neurons expressing a fluorescent-tagged synaptophysin. They found that vGlut1 transporter activity is necessary for proton efflux in resting synaptic vesicles.

Taken together the results provide an explanation for why brain tissues have a higher resting energy expenditure than other tissues and implicate proton efflux in glutamatergic synaptic vesicles with VGlut and V-ATPase as key mediators.

Location, location, location: Zapping inflammation with site specific electroacupuncture.

One idea to explain successful outcomes with acupuncture in human disease is the notion of meridian channels linked to acupoints at specific body regions. These meridian channels have not been identified anatomically but are thought to relate functionally to somatosensory autonomic reflexes. Liu et al. provide evidence for a structure-function link between specific electroacupuncture sites and the vagal-adrenal axis, further demonstrating that stimulation with defined protocols at specific acupoints can modulate the inflammatory response in mice.

Shenbin Liu, Zhifu Wang, Yangshuai Su, Lu Qi, Wei Yang, Mingzhou Fu, Xianghong Jing, Yanqing Wang, Qiufu Ma. A neuroanatomical basis for electroacupuncture to drive the vagal–adrenal axis. *Nature*. 598, 641-645 (2021). https://doi.org/10.1038/s41586-021-04001-4.

The notion of a high degree of acupoint specificity has long been controversial in the field of acupuncture, partly due to the absence of direct neuroanatomical evidence. A vagal-adrenal axis Zusanli (ST36) acupoint has been reported recently that can be controlled by electroacupuncture stimulation (ES) in the hindlimb, but not by ES of the Tianshu (ST25) abdominal acupoint.

To test the hypothesis that sensory pathways in the hindlimb drive somato-vagal-adrenal reflexes distinctly from abdominal pathways Liu et al. generated mice that express the tdTomato reporter gene in a subset of dorsal root ganglion sensory neurons (PROKR2^{Cre}) that innervate deep limb tissues but not skin epidermis. They demonstrated that PROKR2^{Cre} neurons were present more frequently at limb levels than thoracic levels and that centrally, PROKR2 neurons innervated mainly the superficial laminae of the dorsal spinal cord. In the periphery, they innervated the deep hindlimb fascial tissues, but not the abdominal fascia (peritoneum). This gave the authors a way to distinguish innervation of the hindlimb ST36 region from the abdominal ST25 region.

To explore the role of PROKR2 neurons in driving the vagal-adrenal axis function ES stimulation (0.5 mA) was delivered to the hindlimb ST36 acupoint. In control mice ES induced neuronal activation markers. However, mice in which PROKR2 neurons expressed the diphtheria toxin receptor (DTR) and which had been injected previously with diphtheria toxin to ablate DRG neurons innervating the periosteum (PROKR2^{ADV}-Abl), the neuronal activity markers were not evident. Therefore, at 0.5 mA ES the data implicate PROKR2 neurons in activation of the vagal-adrenal axis.

Activation of the vagal-adrenal axis can suppress systemic inflammation induced by the bacterial toxin lipopolysaccharide (LPS) and increase serum levels of the pro-inflammatory cytokines tumour necrosis factor (TNF) and interleukin-6 (IL-6). When LPS-treated mice received 0.5 mA ES at the ST36 site TNF and IL-6 levels were reduced by 50% and survival was improved compared to their sham ES treated littermates. These effects were not observed in PROKR2^{ADV}-Abl mice, implicating PROKR2 neurons in the response.

Interestingly, gain of function studies using optogenetic stimulation of PROKR2^{Cre} neurons demonstrated that stimulation through the ST36 site is sufficient to drive the vagal-adrenal axis, but not sympathetic reflexes. Collectively, this work demonstrates evidence for the selectivity of and specificity for electroacupuncture points and suggests the potential for electroacupuncture to be targeted to drive specific autonomic responses relevant to disease states, including cytokine release syndromes. In some cases, cytokine storms have been linked with poor outcome in Covid-19 related illness, so this may suggest ES as a potential future therapy.

Off with its head: A role for potassium ion transport in hydra budding and head regeneration?

With a few exceptions, human body parts do not regenerate spontaneously, but this is possible for some other animals, most commonly invertebrates and lower vertebrates. For example, the aquatic cnidarian, *Hydra* develops bud structures spontaneously during asexual reproduction and if it loses its head a *Hydra* can grow a new one. However, it is not clear whether the same repertoire of genes is employed for budding and regeneration in *Hydra*. Murad et al. mapped the transcriptional dynamics and mode of regulation of both processes, revealing immense complexity and hinting that potassium ion transport may be involved.

Rabi Murad, Aide Macias-Muñoz, Ashley Wong, Xinyi Ma, and Ali Mortazavi. Coordinated Gene Expression and Chromatin Regulation during *Hydra* Head Regeneration. *Nature*. 598, 641-645 (2021). https://doi.org/10.1038/s41586-021-04001-4.

Anatomically, an adult *Hydra* consists of four regions: the foot, the budding zone, the body column, and an apical head region comprising tentacles and a hypostome. The hypostome contains a head organizer region of about 50-300 cells that signals neighbouring cells to adopt appropriate tentacle or hypostome identities. The precise nature of these signals is not established, but canonical Wnt signalling plays a central role in both budding and head regeneration.

The authors used RNA-seq techniques on tissues isolated from different adult *Hydra* body tissues (tentacles, hypostome, body column, budding zone and foot) to characterize the genes upregulated in the hypostome. They found that the foot, tentacle and hypostome were more distinct from each other than the body column and budding zone. Gene ontology term enrichment analysis for genes upregulated uniquely in each tissue revealed that the body column and budding zone had no significant enrichment. However, genes in the tentacle were enriched for G protein coupled receptor signalling, protein glycosylation, potassium ion transport and cell adhesion. Genes uniquely upregulated in the hypostome included those related to G protein coupled receptor signalling, potassium ion transport, multicellular organism development and Wnt signalling.

When considering the dynamic time course of gene expression, a set of 298 differentially expressed genes were identified during the 48-hour head regeneration and 72-hour budding time courses. The authors hypothesized that genes upregulated in the hypostome during episodes of regeneration or budding would identify key genes for head patterning, but the analysis failed to show differences, probably because such genes are constitutively active to maintain head tissue identity. Principal component analysis of the time course of head regeneration and budding revealed distinct trajectories that eventually converge, with sets of genes specific or common to both regeneration and budding. Further analysis in the context of head regeneration identified 27,137 open-chromatin elements that are open in one or more sections of the organism's body or regenerating tissue; ~37% were promoter-like and ca. 11% were enhancer-like elements.

Murad and colleagues have shown that during *Hydra* budding and regeneration a complex set of genes are active in distinct spatial and temporal contexts. *Bioelectricity* readers will likely note that potassium transport was identified in the screens. Considering the established roles for ion transport in driving important processes in other systems (some highlighted in this instalment of *Bioelectricity* Buzz) it seems an area ripe for research.

Which way is up? Proton flux controls auxin-driven differential growth of plant roots and shoots.

Plant roots and shoots grow in opposite directions in response to gravitational cues and under control of the phytohormone, auxin. Li et al. explored the influence of proton transport on auxin signalling in plant roots, unveiling counteracting mechanisms that converge on H^+ flux that control root growth dynamics.

Lanxin Li, Inge Verstraeten, Mark Roosjen, Koji Takahashi, Lesia Rodriguez, Jack Merrin, Jian Chen, Lana Shabala, Wouter Smet, Hong Ren, Steffen Vanneste, Sergey Shabala, Bert De Rybel, Dolf Weijers, Toshinori Kinoshita, William M. Gray, Jiří Friml. Cell surface and intracellular auxin

signalling for H^+ fluxes in root growth. Nature. 599, 273-277 (2021). https://doi.org/10.1038/s41586-021-04037-6.

Auxin is a plant hormone that regulates growth oppositely in shoots, which grow upward and in roots, which grow down. In shoots, intracellular auxin Transport Inhibitor Response1 and Auxin-Signalling F-Box (TIR1/AFB) receptors activate H^+ pumps by downstream transcriptional regulation. According to the acid growth theory, acidification of the intercellular space (apoplast) promotes cell elongation and growth of shoots. In roots auxin inhibits growth through a non-transcriptional route involving the TIR1/AFB receptor in a way that is mechanistically distinct from that in shoots. The cell surface protein Transmembrane Kinase1 (TMK1) is involved in growth, but its role in auxin signalling in root growth is not resolved. Therefore, Li and colleagues explored the antagonistic actions of TIR1/AFB and TMK1 auxin signalling in regulation of root growth dynamics to unpick these opposing mechanisms.

Arabidopsis thaliana roots labelled with a membrane impermeable ratiometric pH indicator were imaged during root tip growth. An apoplastic pH gradient was observed, with pH decreasing from the transition zone to the elongation zone and upon addition of auxin the apoplastic pH increased. Use of an intracellular pH reporter showed a rapid cytosolic pH decrease concomitant with the apoplastic alkalinization, suggesting H^+ efflux across root cells upon auxin treatment, which was confirmed using non-invasive microelectrodes.

Manipulation of apoplastic pH (exchanging the growth medium of pH 5.8 to pH 6.1) immediately halted growth, but return to more acidic medium restored growth, demonstrating a causal relationship between root apoplastic pH and growth. Furthermore, it suggested that the auxin-triggered alkalinization of the apoplast is the likely mechanism for growth arrest. The pH changes observed were too rapid to involve transcription, so attention turned to plasma membrane H^+-ATPases, including ATPase2 (AHA2) that were known to be phosphorylated upon auxin addition. AHA2 in roots was phosphorylated rapidly upon auxin addition, causing H^+-pump activation and suggesting that it acts antagonistically to the apoplast alkalinization triggered by auxin.

To explore how apoplastic pH and AHA relate to auxin driven growth the authors verified that AHA2 interacted with the cell surface protein TMK1 and then used phosphoproteomic analysis to verify that TMK1 mediates auxin-stimulated phosphorylation and activation of AHA2.

Next the mechanism was sought for the opposing apoplast acidification (by TMK1 phosphorylation and subsequent activation of plasma membrane H^+ ATPAses) and the apoplast alkalinization (observed upon auxin treatment). Auxin influx transporter mutations were exploited to demonstrate that intracellular auxin perception is required for apoplast alkalinization. Experiments using roots with mutations in TIR1/AFB receptor signalling pathway confirmed participation of intracellular TIR1/AFB receptors in auxin-induced apoplast alkalinization.

Plant roots navigate complex 3-dimensional soils, changing course rapidly to avoid obstacles, but how this is regulated has been a long-standing question. Li and colleagues demonstrated elegantly that cell surface based TMK1 activates H^+ pumps and that intracellular TIR1/AFB signalling causes net cellular H^+ influx. These auxin-triggered mechanisms converge on regulation of extracellular pH to determine whether root growth is active or inhibited.

Shape shifter: Catching the voltage gated channel in the act.

How voltage-sensing domains in cell membranes regulate channel gating is difficult to resolve from structures because they tend to adopt an activated state in artificial systems, which lack a membrane potential (e.g. detergent micelles). *Arabidopsis thaliana* vacuolar membrane channels (AtTPC1) were explored by Yea and colleagues to elucidate the mechanism of action of the voltage-sensing domain of a voltage-gated Ca^{2+}-modulated two pore cation channel (AtTPC1).

> Fan Yea, Lingyi Xua , Xiaoxiao Li, Weizhong Zeng, Ninghai Gan, Cheng Zhao, Wei Yang, Youxing Jiang, Jiangtao Guo. Voltage-gating and cytosolic Ca^{2+} activation mechanisms of Arabidopsis two-pore channel AtTPC1. PNAS. 118(49) e2113946118 (2021). https://doi.org.

At hyperpolarizing membrane potentials most voltage-gated ion channels (VGICs) are stabilised in the resting state, with the channel gate closed. Depolarization of the membrane potential is detected by the voltage-sensing domain (VSD) of the VGIC, triggering a conformational change that opens the channel gate, activates the VGIC and initiates downstream signalling.

Yea et al. studied AtTPC1, a two-pore nonselective cation channel that generates the plant slow vacuole current. The group previously determined its crystal structure in the closed state and found that only the second subunit within the VSD (VSDII) senses membrane potential and adopts a resting state. The helix-loop-helix structure (EF hand 2) in AtTPC1 that binds Ca^{2+} is important for Ca^{2+}activation, with Ca^{2+} binding stabilising VSDII in a resting state. Both membrane potential depolarization and cytosolic Ca^{2+} are needed to activate AtTPC1 but Ca^{2+} inhibits the channel from the luminal side as it binds to and stabilizes VSDII in the resting state (similar to hyperpolarizing membrane potential). The contrary effects of Ca^{2+} make it difficult to stabilize the biochemically purified AtTPC1 channel in the activated state.

The authors proposed that conformational changes in VSDII triggered by membrane potential and those triggered by Ca^{2+} binding to the cytosolic EF domain are coupled to different transmembrane segment domains. The notion of multiple stimulus (membrane potential and Ca^{2+}) gating was therefore tested using cryoelectron microscopy to determine the AtTPC1 structures in open and partially closed conformations under different Ca^{2+}conditions.

Cryoelectron microscopy revealed AtTPC1 structure at 2.8 - 3.3 Å resolution, largely agreeing with the previously determined crystal structure and permitting a complete model of the voltage sensing VSDII. Comparison of structures under 1 mM Ca^{2+} (to mimic a Ca^{2+} -activated state) and 50 mM Ca^{2+} (to mimic a Ca^{2+} -inhibited, closed state) conditions revealed significant structural differences in key gating components, including the EF-domain, VSDII and the pore domain. Coupled with experimental mutagenesis at the vacuolar luminal Ca^{2+} inhibitory site the data demonstrate AtTPC1 structures in closed conformation with resting VSDII and an unbound EF-hand domain, and in a partially open conformation with activated VSDII and a Ca^{2+}-activated EF-hand domain.

Evidence for the mechanism of cytosolic activation and its coupling to voltage-gating clarifies how different stimuli are integrated to gate the channel. AtTPC1 approximates a classical VGIC in terms of voltage-sensing machinery, so the insights gained here may extend to many VGICs in a wide range of cellular systems.

A WINDOW of opportunity: A wireless DNA-based biosensor to detect early stage wound infection.

Chronic wounds are a substantial burden in clinical settings, with bacterial infection contributing to poor healing outcome. Identifying and tackling infection at an early stage improves outcome but identifying pathogenic bacteria with culture-based testing delays appropriate wound care. Xiong and colleagues have developed a DNA-based hydrogel sensor they call WINDOW that responds selectively to pathogenic bacteria, sending a wireless alert to a smartphone, even before signs of infection are visible at the wound.

> Ze Xiong, Sippanat Achavananthadith, Sophie Lian, Leigh Edward Madden, Zi Xin Ong, Wisely Chua, Viveka Kalidasan, Zhipeng Li, Zhu Liu, Priti Singh, Haitao Yang, Sascha P. Heussler, S. M. P. Kalaiselvi, Mark B. H. Breese, Haicheng Yao, Yuji Gao, Kavitha Sanmugam, Benjamin C. K. Tee, Po-Yen Chen, Weiqiang Loke, Chwee Teck Lim, Grace Shu Hui Chiang, Boon Yeow Tan, Hao Li, David Laurence Becker, John S. Ho. A wireless and battery-free wound infection sensor based on DNA hydrogel. Sci. Adv. 7, eabj1617 (2021). https://doi.org.

Xiong et al. have developed a flexible, tuneable, DNA-based hydrogel (DNAgel) that can be applied to wound dressings. When DNAgel is degraded by DNase enzymes associated with specific bacteria it dissolves the hydrogel, changing the dielectric permittivity near integral electrodes and modulating their capacitance, which triggers a radio frequency response. A signal transduction interface at the capacitive sensing electrodes comprises a resonant (LC) tank and a half-wave rectifier that converts the capacitance signal into a voltage output. The resonant frequency of the LC signal is set at 13.56 MHz to permit near field communication (NFC) with a smartphone. The authors call this system 'wireless infection detection on wounds' (WINDOW).

The system was first tested *in vitro* over 48 hours by attaching WINDOW to a gauze pad soaked with supernatant (contains DNase) collected from *Staphylococcus aureus* cultures grown to various concentrations and then recording the resulting signal with a smartphone. WINDOW detected *S aureus* at levels near the low end of clinical infection thresholds (10^5 colony forming units- CFU) but at high concentrations (>10^7 CFU) it saturated the sensor because it degraded the DNAgel completely.

WINDOW was then placed on acute full thickness wounds in mice that also had gauze saturated with either live *S. aureus* or sterile tryptic soy broth (control) attached to the wound site. At 24 hours signals from uninfected wounds and control wounds were indistinguishable. However, wounds treated with 10^5 or 10^6 CFU of *S aureus* showed a signal change of 0.4 V in 24 hours, which triggered an alert on a smartphone brought into the vicinity of the wound.

The technology is impressive in that it is wireless, battery free and tuneable to multiple scenarios. Here the sensor distinguishes between pathogenic bacteria and non-harmful commensal bacteria found naturally in the skin microbiome, only triggering an alert for the pathogens. This has obvious benefit for rapid treatments of wound infection but the DNAgel and WINDOW technology also have the potential to be modified to detect other species and to be integrated with other sensing technologies that could broaden its utility beyond wounds.

And that's the *Buzz* for now. I hope it's inspired you and that you learn as much from reading it as I learned while putting it together.

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