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Review:

Additional Proposed Tests of the Soliton/Wave-Action Potential Model, and How the Thermodynamic/Theory-Based Philosophical Approach Abandons the Scientific Method

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Abstract

This article is a followup to an earlier review which outlined some of the interesting features of the soliton/wave-action potential (AP) model, and noted the need to test its key aspects; including the need to test if its presumed lipid phase transition is actually happening during AP firings in excitable cells. The intent here is to point out the sort of tests, and evidence from them, that might be needed if the soliton/wave-AP model is to be accepted broadly by biologists. Here, after an overview of the modern electrophysiological-AP model and of the soliton/wave-AP model, there are three areas considered. First, possible compositional influences on membrane properties relative to the soliton/wave-AP model are presented. Including questions with regard to the soliton/wave-AP model's assumption that changes in surface potentials influence the transmembrane potential. Second, some recent work from the good folks who advocate for the soliton/wave-AP model concerning the occurrence of lipid phase transitions in neurons or in extracts from nervous tissues are examined. Here it is noted that there is a need to consider whether these lipid phase transitions happen within normal physiological conditions or not. Third, and finally, the advocates for the soliton/wave-AP model have adopted a thermodynamic/theory-based philosophical approach in their studies. It is argued that this philosophical approach is a radical departure from the philosophical approach used under the scientific method. The features of this new approach, and implications its use, are examined.

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1. Introduction.

The modern electrophysiological-action potential (AP) model has stood the test of time. But within the last two decades a new model that attempts to account for action potential production in excitable cells has been put forward; the soliton/wave-AP model (Heimburg et al. 2005). This new soliton/wave-AP model is creative and certainly offers a different perspective of how an action potential might be produced. Such new thinking is welcome as it offers new ways to examine old phenomena. But for this new model to be accepted it needs to undergo new and critical tests which bring forward new data showing that it can account better for existing knowledge than the current highly accepted electrophysiological-AP model. So, building on previous comments (Meissner 2018), this article will suggest some more of the sorts of tests of the soliton/wave-AP model that might yield some information helpful in terms of discerning whether or not the soliton/wave-AP model lives up to the claims its advocates make for it.

The main points to be made in this article are several. First, given that in biological membranes there is such broad compositional diversity seen, especially compared to simple phospholipid bilayer systems, that the claims made by the advocates for the soliton/wave-AP model, that their relationships are universal and apply across all membrane types and compositions, may be called into question. Examples of situations in bilayers and membranes in which the need to test if soliton/waves can be produced in such settings will be indicated. Also the assumption made by the soliton/wave-AP model that surface potential changes can account for the membrane potential shifts we see during an AP will be examined. Second, in making claims that lipid phase transitions are commonly seen in biological membranes the good people who advocate for the soliton/wave-AP model have not yet produced compelling evidence to back up this claim. How some of the evidence they have recently presented on this issue falls short will be reviewed, and suggested improvements in the methods used to examine whether or not lipid phase transitions are common and adaptive in biological membranes will be offered. In addition, how some of the findings from such recent studies need to be evaluated in terms of whether or not they occur under normal physiological conditions will be outlined. Third, the new thermodynamic/theory-based philosophical approach that the advocates of the soliton/wave-AP model put forward will be examined, and its features described. Examples of its use will be presented, and how it differs from the standard scientific method's philosophical approach noted. The vast majority of scientists today expect that models will be evaluated from within the scientific method,

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and so the different standards of evidence being used under this new thermodynamic/theory-based philosophical approach may not come across as convincing to many scientists. It is suggested that the scientific method is still the best philosophical approach that science has devised, and so we should not replace it with another philosophical approach.

1a. The modern electrophysiological-AP model.

Since it is both a well established model, and is the logical alternative to the proposed soliton/wave-AP model, it is perhaps appropriate to present a brief review of the modern electrophysiological action potential model. This electrophysiological-AP model is derived from the earlier work of Hodgkin and Huxley (1952a), but this modern model has several significant modifications which build upon and greatly extend that earlier work which makes up the classical Hodgkin-Huxley-AP model. Such extension is natural when working with empirical models. Thus, while much of the experimental findings reported by Hodgkin and Huxley, and others, are still important under the current electrophysiological-AP model, many new features have been added, for instance more refined quantitative empirical equations and software systems have been derived (Platkiewicz et al. 2010; Paci et al. 2012; Kolaric et al. 2013; McDougal et al. 2013; Ma et al. 2017a; Kisnieriene et al. 2019; Cohen et al. 2020). Let us, then, start with a simple statement of the narrative of this electrophysiological-AP model:

> If there are electrochemical gradients for two different ion types each in the proper orientation across the plasma membrane, then energy to power movement of each of these ion types across the membrane is available. If one type of voltage-gated ion channel is activated by a mild membrane potential depolarization (caused by events at the post-synaptic membrane of the neuron upon neurotransmitter detection), then the first type of ion channel opening is induced which alters the permeability of the membrane and this first ion type can move down its energy gradient. As these ions of the first type cross the membrane, then the charges being carried by these ions will induce a change in the membrane potential leading to the initial part of the action potential's membrane potential shifts. If a second type of voltage-gated ion channel is present and is sensitive to this change of the membrane potential to more positive values due to the movement of the first ion, then this second type of ion channel will open and that will alter the permeability of the membrane to a second ion type. This will be seen as a change in the membrane permeability to this specific ion type during this part of the action potential. As this second ion type moves across the membrane, down its energy gradient, it carries charge and so also

contributes to changes in the membrane potential. The movement of this second ion type will cause the membrane potential to return to a negative state, and if the channels are open long enough a hyperpolarization beyond the normal resting potential may occur. Each of these channel types must deactivate in a brief period, and so return to their respective initial states over time, and the membrane potential then returns to its resting state. Notice that the flow of these ions down their respective electrochemical gradients will generate heat, and over the long term and with many action potential firings the combined flow of ions will eventually dissipate their respective transmembrane electrochemical gradients. This indicates the need for ion transport systems to be used to maintain these energy gradients and these transport systems are operated at metabolic cost.

The reader should notice several features of the above narrative of the electrophysiological-AP model: First, while the flow of two ion types is indicated, the specific ions involved is not designated in the above narrative. This is because work has shown that in different cells and in different species the production of action potentials can be achieved by the flow of different combinations of ions in different directions (Osterhout 1934; Tasaki et al. 1962, 1965, 1966; Kitasato 1968; Homann et al. 1994; Fromm et al. 2007; Iosub et al. 2015). The fact that the same action potential phenomenon can be produced in different contexts in such multiple ways using different ions and ion channel types may be taken as confirmation of this model in terms of its broad features as it has apparently evolved multiple times in various lineages. Second, this electrophysiological AP-model notes the use of voltage-sensitive ion channels to alter the permeability of the plasma membrane to each specific ion type (Thiel et al. 1997; Roux 2017; Powell et al. 2021). This relates to the classic observation that during action potentials there is a change in the membrane permeability to specific ion types, with the subsequent ion flows resulting in the AP. This change in permeability was well documented when Hodgkin and Huxley presented their outline for an action potential (Hodgkin 1937a, 1937b; Hodgkin et al. 1939, 1945, 1949, 1952a, 1952b, 1952c, 1952d, 1952e, 1955), and since then the role of specific protein-based ion channels of various types operating in the plasma membrane in different cell types to achieve this AP pattern of change in membrane permeability has been established (Bean 2007; Liu et al. 2012). The role of the ion channels in AP generation has been shown to be essential, as illustrated by the work of Shapiro et al. (2012) who found that cultured oocytes that do not normally display action potentials, can do so after being transformed with the genes for the needed Na^+ and K^+ voltage-gated channels and upon their proper expression and the deployment of these proteins to the oocyte's plasma membrane. The essential nature of these ion channels is also indicated by disease states being associated with the lack of proper

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expression of genes coding for them. Indeed, changes in the expression of a gene coding for one ion channel is reported to occur with age, altering AP firing rates and the quality of sleep (Li et al. 2022). Also the application of stress to the plasma membrane of neurons has been reported to alter ion channel activity and so alter some features of the resulting AP (Bianchi et al. 2019). A third feature of the above narrative of the electrophysiological-AP model to consider is the flow of each ion type down its specific energy gradient across the plasma membrane (Homann et al. 1994). The two major contributors to this energy gradient are, first, the membrane potential itself which acts on all ions, and, second, the concentration gradient for each individual ion type across the neuron's plasma membrane. The existence of both the membrane potential and the concentration gradients of the ions involved in a specific action potential have long been established (Keynes et al. 1965; Beilby 2007). These ion electrochemical potential gradients are maintained at high metabolic cost (den Hertog et al. 1969; Ritchie 1973; Mink et al. 1981; Verkerk et al. 1996; Street 2020; Attwell et al. 2001; Magistretti et al. 2015; Kann 2016; Jensen et al. 2020). Indeed the neurons in the central nervous system are at such risk of damage from reactive oxygen species that form with the high metabolism present there that neighboring astrocytes pass them antioxidants to help them avoid damage (Bélanger et al. 2011). These and many other aspects of the above outline of the electrophysiological-AP model have been tested and confirmed in various ways by a truly vast number of studies. Space does not allow a full listing of all of such studies, but those interested in more of this evidence and its context might examine the following, and the items cited within them (Nathan et al. 1962; Watanabe et al. 1967; Meves et al. 1973; Pickard 1973; Tasaki 1982; Fernández et al. 1983; Hille 1984; Shepherd 1988; Wayne 1994; Huxley 2002a, 2002b; Johnson et al. 2002; Beznilla 2006; King et al. 2014; Peyrard 2020; Street 2020; Cornejo et al. 2022), and for how APs fit into the history of the study of biological membranes see the review by Lombard (2014). Thus there is much well established empirical evidence that supports this model, and upon which acceptance of this model is currently based.

The modern electrophysiological-AP model notes the role of the electrochemical gradients for specific ions, and the changes over time in specific ion permeabilities of the membrane can lead to changes in the transmembrane potential (Powell et al. 2021). These two aspects are often considered in electrophysiology via the Nernst potential equation, and the Goldman-Hodgkin-Katz equation (Nobel 1974).

The Nernst potential equation is:

$$E_{Nj} = \left(\frac{R\,T}{z_j\,F}\right) \ln\left[\frac{a_{jo}}{a_{ji}}\right] \qquad \text{(eq. 1)}$$

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Where: E_{Nj} is the Nernst potential for ion j. R is the gas law constant. T is the temperature in degrees Kelvin. z_j is the charge carried by ion j. F is the Faraday constant. And a_{jo} and a_{ji} are the activities across the plasma membrane of ion j (usually approximated by using their concentrations) in the bulk solution at the outside surface of the membrane versus the bulk solution at the inside face of the membrane respectively. The Nernst equation is derived directly from the law of conservation of energy (Nobel 1974; Hille 1984), and indicates for a given ion type the energy in the membrane potential (E_{Nj}) that would be needed to directly counter the opposing energy in a concentration gradient of ion j across the membrane. Comparison of the Nernst potential for a given ion type to the actual transmembrane potential allows an estimate of which direction that ion type will move if the membrane becomes permeable to that type of ion.

This raises the issue of changes in membrane permeability, which is addressed in the Goldman-Hodgkin-Katz (GHK) equation:

$$E_m = -\left(\frac{RT}{F}\right)\ln\left[\frac{(P_K[K+]_o + P_{Na}[Na+]_o)}{(P_K[K+]_i + P_{Na}[Na+]_i)}\right] \qquad \text{(eq. 2)}$$

The R, T, and F terms are as given above. Here the ions K^+ and Na^+ are considered in terms of their outside and inside concentrations. This GHK equation can be expanded to include other ions whose movement across the membrane may also contribute to the final membrane potential (E_m). For instance, Cl^-, Ca^{+2}, or H^+ are sometimes added for certain cell types or in some species (Kitasato 1968; Nobel 1974) when they contribute significantly. Notice that each ion type has a permeability coefficient (P_K and P_{Na}) associated with it. The permeability coefficients here are dynamic, and so can change rapidly over time as is found in the study by Takashima (1979), and this leads to changes in transmembrane potential we call an action potential.

With ion flow across the membrane the law of conservation of charge has some important influences on the system. The extent to which the transmembrane potential is altered with ion flows is influenced by the resistance of the membrane, and the speed of its alteration is influenced also by the capacitance of the system. Thus, the reason in classical work for adoption of cable theory (Tasaki 1982), and for the use of analogies to electronic circuits (Hodgkin et al. 1952a), follows directly from the established finding that ions are moving across the membrane during an action potential. To ignore the consequences of such charge movements, then, would be to ignore the consequences of the law of conservation of charge.

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The focus of this model is not only on membrane resistance or membrane potential alone, but changes in capacitance during an AP have also been examined. Fozzard (1966) modified the equations presented earlier by Hodgkin and Huxley (1952a) to account, in an excitable muscle cell, for the changes in capacitance seen with AP firings. Palti et al. (1969) report how with AP firing there is a change in the capacitance of the squid giant axon, and notes how this fits into the electrophysiological-AP model. The Sabah et al. (1972) article is an example of an exploration of altering the parameters used in the Hodgkin and Huxley equations from 1952, including how changes in capacitance would influence the features of the action potential. Chiu et al. (1982) notes how in frog nerve internode regions there is a change in capacitance seen in association with the K^+ current during APs. The Fernández et al. (1983) article notes the changes in capacitance seen during APs in squid giant axons and alters the classical Hodgkin-Huxley empirical equations to take this into account. The Jurisic (1987) and the Akemann et al. (2009) articles discuss how the protein conformational changes that occur during an AP can lead to a rise in the membrane capacitance. The influence of the shifts of the voltage-sensing domains of membrane proteins during an AP on the transient changes in capacitance of the neuron have been modeled by Kim et al. (2016). Sangrey et al. (2004) modified the Hodgkin-Huxley equations to take into account such a capacitance shift during an AP, an alteration they argue is justified as the Hodgkin-Huxley equations were empirically derived and so are open to modification based on new evidence. The Franzen et al. (2015) article gives a nice description of how across the development of a neuron there are changes in the cell's membrane resistance and capacitance and notes how this leads to alterations in the features of the AP that are observed at different points of development. Gulledge et al. (2016) modeled how morphological changes occur in neurons, and include in their analysis consideration of how this can lead to changes in cell capacitance and so influence AP features. The Jerusalem et al. (2019) review notes how with AP passage protein conformational changes can lead to local transient changes in capacitance. Varela et al. (2021) notes the AP models recently used to account for mechanical stress-induced changes in capacitance in cardiac tissue. Thus, along with monitoring changes in the membrane resistance to various ion types and membrane potential, the modern electrophysiological-AP model also considers changes in membrane capacitance that arise during APs as well. This indicates that the original Hodgkin-Huxley AP equations (Hodgkin et al. 1952a), which assumed a constant capacitance in the neuron during AP firing, have been extended from that earlier form to give us the current electrophysiological-AP model in which capacitance changes are acknowledged and incorporated into the model. This point is raised as there are those who claim that the current modern electrophysiological-AP model somehow does not account for changes in capacitance, or that it either assumes or somehow requires a constant capacitance, during AP firing (Heimburg 2021; Carrillo et al. 2022). Such arguments would seem to be directed at the seventy year old Hodgkin-Huxley AP equations, and apparently are

 doi:10.20944/preprints202208.0248.v1

ignoring the modern updates that have been made to the model and its equations since that time?

While the above narrative has provided a path to examine how the membrane potential changes associated with an action potential are produced, there are other phenomena that are reported to be associated with action potentials. It has been suggested that some of these phenomena may be accounted for as being due to the large change in transmembrane potential of the action potential acting on dipole molecules (*i.e.*, proteins and lipids) in the membrane, and so causing secondary effects (Ueda et al. 1974; Das et al. 1995; El Hady et al. 2015; Ling et al. 2020). Shrager et al. (1987) note that changes in optical properties occurred in a neuron in sync with the timing of the action potential and in proportion to the changes in the membrane potential. This view is supported by the fact that several of these secondary effects have been shown to arise to some extent when a neuron is placed under a hyperpolarizing clamp voltage (*i.e.*, from the resting potential of about -70 mV the voltage is moved to roughly -120 mV). Such a hyperpolarized clamp voltage does not typically induce an action potential, but it does induce some of the sort of secondary effects commonly reported to be associated with APs. Thus, there are several reports both for non-neuronal cells in culture (Oh et al. 2012) and for neurons (Cohen et al. 1971; Tasaki 1982) that such a hyperpolarization of the plasma membrane can induce changes in the absorption of light by, or of other optical properties of, the membrane. While Lee et al. (2017) reported that a plasma membrane hyperpolarization can lead to changes in the Raman scattering spectrometric data obtained from neurons. In addition, Terakawa (1985) reports that such a membrane hyperpolarization can induce a small shift in a neuron's internal pressure. In a more indirect manner, Howarth et al. (1968) noted that in mammalian nerves alteration of the external Na^+ concentration both alters the amplitude of the voltage swing seen during an action potential and alters the amount of initial positive heat produced, making it possible that the amplitude of this membrane potential shift during the AP may influence the magnitude of the heat generated. It would be interesting to examine if an imposed hyperpolarization on the plasma membrane of neurons might also have other effects, for instance would this alter the proportion of lipid rafts present in the membrane during AP passage? For more on how some of these AP associated phenomena may fit under the electrophysiological-AP model see the previous review by Meissner (2018).

1b. The soliton/wave-AP model.

The soliton/wave-AP model has been outlined before (Meissner 2018). The version of this model that is considered here is based on work done with phospholipid bilayers and monolayers by the lab groups of Heimburg (Andersen et al. 2009; Appali et al. 2012; Ebel et al. 2001; Gonzalez-Perez et al. 2014, 2016; Heimburg 1998, 2009, 2012,

 doi:10.20944/preprints202208.0248.v1

2019, 2021; Heimburg et al. 2005, 2007; Laub et al. 2012; Lautrup et al. 2011; Mosgaard et al. 2015a, 2015b; Mužić et al. 2019; Schrader et al. 2002; Wang et al. 2017, 2018; Wunderlich et al. 2009; Zecchi et al. 2017, 2021), and of Schneider (Fabiunke et al. 2021; Fedosejevs et al. 2022; Fichtl et al. 2016, 2018; Fillafer et al. 2013, 2016, 2017, 2018, 2021, 2022; Griesbauer et al. 2009, 2012; Kang et al. 2020; Kappler et al. 2017; Mussel et al. 2017, 2019a, 2019b, 2021; Schneider 2020, 2021; Schneider et al. 1999; Shrivastava et al. 2013, 2014a, 2014b, 2018a, 2018b; Steppich et al. 2010). These workers have used observations and experimental findings from these lipid bilayer and monolayer systems to derive biophysical relationships largely based on thermodynamic principals and various conservation laws. They then argue that these relationships are universal, and so would apply both to lipid bilayers and to biological membranes broadly irrespective of specific molecular details (Fillafer et al. 2021; Schneider 2021), and they claim that these relationships can be applied without knowledge of membrane composition (Schrader et al. 2002). A brief narrative of how their model proposes to account for the pattern of changes in membrane potential we call an action potential in excitable cells would go roughly as follows:

> In a biological membrane the lipids are induced to undergo a lipid phase transition. This would be something like a rapid reversible transition from a lipid liquid-crystalline phase to a gel phase. During such a reversible transition there would be alteration of the membrane density, thickness, optical properties, and alteration of the display of surface charges locally. This change in the surface charge density during the lipid phase transition results in a change in the local surface potential, and if this change in surface charge density differs between the two faces of the biological membrane then a difference in surface potential will result between the two faces. This difference in surface potential changes between the two membrane faces during the reversible lipid phase transition is then proposed to account for the membrane potential changes we define as an action potential.

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Table 1. The equations in articles on the soliton/wave model in lipid bilayers and monolayers that make use of the changes in specific heat capacity seen during lipid phase transitions.

Reference:	Equation numbers in each reference that use specific heat capacity changes (ΔCp) in calculations:
Ebel et al. (2001)	2, 3, 12, 14
Schrader et al. (2002)	1, 3, 4, 5
Heimburg et al. (2005)	2, 3
Griesbauer et al. (2009)	5b
Steppich et al. (2010)	0, 5c, 7, 8, 9, 10
Mužić et al. (2019)	1, 5, 6
Heimburg (2019)	8-12
Mussel et al. (2019a)	2.5, 2.9
Mussel et al. (2019b)	4, 6
Mussel et al. (2021)	1, 2

The actual equations used for quantitative analysis under the soliton/wave-AP model differ somewhat depending on the aspects of the features of the soliton/wave that are being estimated; for instance, the velocity it would have, or changes in local denisty of the lipid bilayer, or other features. Of special interest is the use of the changes in specific heat capacity (ΔCp) associated with the lipid phase transition which is so essential for the soliton/wave-AP model. Table 1 indicates the equations presented in specific articles that make direct use of changes in the specific heat capacity seen during lipid phase transition. Changes in specific heat capacity are typically estimated by the use of differential scanning calorimetry, and integration of ΔCp over a range of temperatures can then give an estimate of the enthalpy of the lipid phase transition (Ivanova et al. 2001; Haynie 2008; Heimburg 2007; Raudino et al. 2011). This use of the specific heat capacity is part of the reason that these workers call this a "thermodynamic approach," and as stated by

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Lautrup et al. (2011, pg. 1) they suggest that: "A primary virtue of a thermodynamic description of pulse propagation in nerves lies in its predictive power. This is a natural consequence of the fact that thermodynamics allows us to establish connections between macroscopic thermodynamic observables without the need for detailed consideration of their microscopic origins." For instance, Schrader et al. (2002) notes that with knowledge of the ΔCp, and so the enthalpy of lipid phase transition, other properties such as the changes in elasticity, compressibility, and also the velocity of sound seen in the lipid bilayer system can be estimated. Thus from the thermodynamic properties and relations found to operate in lipid monolayer and bilayer systems, this soliton/wave-AP model claims to be able to extend these relations to account for the features of action potentials seen in neurons and other excitable cells.

2. Compositional influences.

This claim of broad application of the soliton/wave equations needs some consideration. This claim has largely been based on data from a liquid crystalline to gel phase transition in homogenous phospholipid bilayers made from just one lipid type (Heimburg et al. 2005). But, when Schrader et al. (2002) suggest that there is no need to consider specific compositions, is it really the case that no matter the composition soliton/waves can be generated? This suggestion that the actual composition of the system does not matter, as the relations put forward are said to apply broadly, and so there is no need to consider the influences of individual molecular details is also stated by others (Shrivastava et al. 2013; Schneider 2021). For instance, Fillafer et al. (2021, pg. 57, original emphasis) states: "The universal character of the phenomenon, *i.e.* the existence of nonlinear pulses in different cells and in simple model membranes indicates that nonlinear excitability *does not depend on specific molecules*." In contrast, others (Sankaran et al. 2020) argue that the properties of a biological membrane are diverse and coupled to differences in composition. The work of Gautier et al. (2013) found that feeding a bacterium different types of fatty acids can induce alterations in the lipid composition of its membrane leading to alterations in its lipid phase transition tendencies across certain temperatures, suggesting that changes in composition may be altering something. Thus the broad claims made by the advocates of the soliton/wave-AP model that composition and specific molecular features do not matter, would seem to, again, be at odds with some reported findings, and so this issue merits our attention.

Therefore, what follows in this section will be a consideration of how the properties of lipid bilayers have been found to have more complexities related to their composition than seems to be considered by the advocates of the soliton/wave-AP model. This will include a consideration of how specific lipid types in homogeneous bilayers show various features, how mixtures of lipids in bilayers can lead to new types of lipid phases and so

 doi:10.20944/preprints202208.0248.v1

new phase transitions between them, how interactions between different lipid molecules can be significant, and then how the interactions of lipids with proteins or with the neutral lipid cholesterol can lead to settings in which the expectations of the soliton/wave-AP model that lipid phase transitions are universal may perhaps not be met. Also a claim of the soliton/wave-AP model is that changes in the surface potential of the faces of a bilayer would produce a change in the macroscopic transmembrane potential, in a pattern we call an action potential; this claim will be questioned. The argument will be made is that composition does matter, that the properties of bilayers that are mixtures, including the highly complex mixtures seen in biological membranes, can be very different from those seen in simple homogeneous lipid bilayers. And so there is a need to test the expectations of the soliton/wave-AP model against various compositions to discover if it is limited to certain compositions, or if indeed the claims of the advocates of the broad applicability of the soliton/wave-AP model are upheld.

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2a. Compositional influences - specific lipid types in pure systems.

In terms of compositional diversity, the membranes of some archaea are reported to have tetraether lipids which span the membrane, creating in effect a cell membrane that is based on a lipid monolayer, and which in some species are reported to show no lipid phase transitions (Chugunov et al. 2014). Other extremophilic archaea, such as *Thermococcus barophilus*, have apolar lipids, such as DPG (2,3-di-0-phytanyl-sn-glycerol), which can make very tough cell membranes to allow them to survive at very high temperatures and pressures (Cario et al. 2015). There are reports that some archaean lipids may show minor lipid phase transitions (Chong et al. 2012). But studies of liposomes produced by extracts from some archaeans show a very low enthalpy of lipid phase transition, and low lipid volume changes and low change in lateral compressibility across their phase transitions relative to DPPC lipid bilayers (Chong et al. 2010). Koga (2012) also notes that some archaeal membranes seem to lack phase transitions over a broad range of temperatures. As the soliton/wave-AP model depends on a lipid phase transition this may bring into question whether such soliton/waves would be able to be produced in such archaeal membranes under physiological conditions? Whether or not the soliton/waves detected during lipid phase transitions found in a DPPC lipid bilayer would then occur in a biological membrane that is based on an ether-linked lipid monolayer, or based on other lipid types from these archean extremophiles, may be an interesting question worth exploring.

In addition, some homogeneous lipid bilayers display multiple phases and phase transitions (Buehler 2016). For instance, the work of Lewis et al. (2007) reports that tetramyristoyl cardiolipin (TMCL) bilayers have a lower temperature lamellar subgel ($L_{c'}$) to gel (L_{β}) phase transition, and a higher temperature L_{β} to lamellar liquid-crystalline (L_{α}) phase transition. Also when the TMCL phosphate groups are fully protonated, or when their charges are well shielded by high concentrations of divalent cations, then a transition from the L_{α} phase to a non-lamellar inverted hexagonal phase can occur. Thus there are three lamellar phases displayed by bilayers made up of this one TMCL lipid, and Lewis et al. (2007) reports differences in the thickness and in average molecular lipid area for these three phases. Also the $L_{c'}/L_{\beta}$ phase transition on heating is found to occur faster than does the phase transition $L_{\beta}/ L_{c'}$ seen when cooling. It may be of interest to examine if all the possible phase transitions between pairs of these phases are each capable of soliton/wave production, and if so whether different features of any resulting soliton/waves are shown to be in correlation with the different phase transitions and with the changing kinetics of transition seen for heating versus cooling for these transitions. Similarly Lewis et al. (1993a) report for saturated phosphatidylethanolamine bilayers with longer (> 18:0) fatty acid chains that at very high temperatures there can be a L_{α} to inverted lamellar (H_{II}) phase transition that can occur, and Lewis et al. (2013) report that FTIR

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spectrometry can be used to detect such H_{II} and other phase states of bilayers. The Lewis et al. (1993b) study notes that when the two fatty acid chains of the phosphatidylcholine used in bilayers are uneven this can lead to a new subtype of gel phase being observed. And Lewis et al. (2000) report for phosphatidyl serine-based bilayers the length of the fatty acid chains used altered the mean transition temperature (T_m) seen, and that bilayers made of this one type of lipid display L_c/L_α, L_c/L_β, and L_β/L_α phase transitions of differing T_ms and enthalpies of transition. Similar findings of multiple phases in pure lipid bilayer systems are also noted by Matsuki et al. (2019). Thus even with bilayers made of just one type of lipid there can be various phase transitions, and it would be of interest to see if all of these different types of phase transitions are able to generate soliton/waves, or if some of them can and others can not. Thus the current soliton/wave-AP model which is based largely on data from DPPC lipid bilayers under going a liquid-crystalline to gel phase transition may need to be tested in these other contexts? If different transitions produce soliton/waves with different features, this might add some complexity to this phenomenon, and raise the question of just which sort of transition(s) should be the focus of our attention when considering biological membranes?

2b. Compositional influences - various types of lipid mixtures.

The soliton/wave-AP model is based largely on a consideration of the liquid-crystalline (L_c) to gell (L_β) phase transition of pure DPPC bilayers (Heimburg et al. 2005). But as noted above even bilayers made from a single lipid type may show other forms of phase transitions, and various studies have found that when certain types of lipids are combined in mixtures to make an artificial bilayer that even more new types of lipid phases arise. Thus changes in composition in bilayer systems have been related to the different types of lipid phases that are found to be produced, and phase diagrams have been published for various lipid combinations (Almeida et al. 1992; Nicolini et al. 2006; Almeida 2011; Kapoor et al. 2011; Konyakhina et al. 2013; Posada et al. 2014; Aghaaminiha et al. 2020). Obviously each of the phase transitions between different phase pairs might be expected to be associated with distinctly different changes in properties. This naturally leads to the question of whether any soliton/waves generated by such phase transitions would all be equal in all their features as well?

In considering some of the relations put forward by Heimburg et al. (2005), Nicolini et al. (2006; pg. 255) comment: "It should be noted that such proportionality relations, like that between enthalpy and volume changes at phase transitions, are probably not universal for order-disorder transitions." One question then is whether the equations relating to the soliton/wave-AP models apply to other phase transitions where the relations between enthalpy and other features may differ, and so Nicolini et al. (2006) questions if the equations presented by Heimburg are truly universal for all types of lipid

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phase transitions. Thus it might not be unexpected that some of these lipid phase transitions might not be well described by the current equations given for the soliton/wave model. (As a poor analogy, one might expect that equations describing the solid to liquid phase transition of water would not work well in any attempt to describe water's transition from a liquid to a gaseous phase state.) Indeed Schrader et al. (2002) notes that some transitions in a 50/50 mol% mix of DMPC/DMG lead to an inverse hexagonal (H_{II}) non-lamellar phase state, and produces properties which they report did not seem to fit well with the soliton/wave equations. This might suggest that the soliton/wave equations used by Schrader et al. (2002) may apply better to bilayer-forming lipids and their combinations, but not so well to phase shifts to non-bilayer states? If so, then it might be reasonable to ask if the other types of lipid phase transitions seen in complex lipid mixtures can all support soliton/wave production equally?

Of special interest would be the transition between the liquid-ordered (L_o) and liquid-disordered (L_d) phase states, as these phases are suggested to be present in the plasma membranes of some eukaryotic cells under physiological conditions (Simons et al. 2000; Jin et al. 2005; Nicolini et al. 2006; Almeida 2011; Kapoor et al. 2011, Raudino et al. 2011; Posada et al. 2014; Sierra-Valdez et al. 2016). As the current soliton/wave-AP model equations are based on a liquid-crystalline (L_α) to gel (L_β) phase transition, we might ask would these equations need modification in order to work well for an L_d/L_o phase transition? Thus the properties of bilayers made up of various compositions, especially those that attempt to mimic the composition of biological membranes, might be worth examining to see if the current soliton/wave-AP equations work equally well in all of them, or if in some cases we might find that the production of soliton/waves is not well supported. But while lipid model systems, such as giant unilamellar vesicles (GUVs), of more diverse composition might be worth studying, Sezgin et al. (2012, pg. 1783) offers the following note of caution: "Inspite of their value for studying membrane phase separation in general, commonly used DOPC/SM/Chol GUVs appear to be rather problematic models to mimic the cell membrane heterogeneity, not only because of their limited complexity, but also due to the seemingly quite different physical nature of the domains."

An exploration of the various lipid phases, and transitions between them, might also be of interest in terms of the specific changes in capacitance that Heimburg (2019) indicates differ with different lipids examined. The current soliton/wave-AP model attempts to account for the rise in capacitance seen during an action potential as being due to a lipid phase transition. One problem with this is found on examining work by Antonov et al. (2003), who report that a transition from a liquid-crystalline to a gel phase results in a thickening of the plasma membrane, as also noted by others (Pagano et al. 1973; Heimburg 1998), but which is associated with a decline in the bilayer capacitance. This

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is the exact opposite of what is seen during an action potential where capacitance rises, so perhaps other phase transitions should be examined? Note that Paiva et al. (2016), using atomic force microscopy, reports that the L_o phase is thicker than the L_d phase, so that any L_d to L_o transition would also involve a thickening of that section of the membrane, and presumably would also result in lowering of the membrane capacitance? Thus while the current soliton/wave-AP model accepts that during an action potential there is a rise in membrane capacitance, which is something that the modern electrophysiological-AP model also accepts, the specific phase transition they use as the basis for the soliton/wave-AP model predicts a decline in bilayer capacitance, as shown by Antonov et al. (2003), but which is seemingly contradicted by the increase in capacitance actually noted during APs. Obviously different transitions between different lipid phases might offer other changes in capacitance than that seen in the liquid-crystalline to gel transition. Thus an exploration of these other phases and of transitions between them would seem to be needed if the soliton/wave-AP model is to account for the rise in capacitance that is known to occur during AP firing? In contrast, while the soliton/wave-AP model attributes the changes in membrane capacitance to a lipid phase transition, the modern electrophysiological-AP model associates this change as being largely due to voltage-dependent protein conformational changes. These two views need not be mutually exclusive, but while there is good evidence for voltage-sensitive protein conformational changes during an action potential (Jurisic 1987; Akemann et al. 2009), whether or not any lipid phase transitions occur during an action potential and whether they are able to account for the increase in capacitance that occurs during an action potential is still not well established.

The advocates of the thermodynamic/theory-based approach have suggested that changes in state should result in distinct properties and functions (Schneider 2020, 2021). Thus one would think that with the finding that in more complex compositional mixtures of lipids more types of lipid phases are produced, representing differences in state, they might be expected to embrace this as an illustration of their argument? Each of these distinct lipid phases presumably have their own distinct properties, and transitions between pairs of these lipid phases might also be assumed to be distinct. Arrais et al. (2007) examined several lipid phase states in bilayer systems and describes how their features differ from each other. Indeed, Almeida (2011) has reported that the free energy change for an L_d/L_o phase transition is different from that from an L_d/gel phase transition, a finding also noted for other phase transition pairs by McMullen et al. (1999). Presumably then the changes in width, thickness, alteration in exposed surface charges, viscosity, etc., of a lipid bilayer might all differ depending on which pair of lipid phases are engaged in a specific phase transition. This would seem to suggest that a study of these phase states and the nature of the transitions between pairs of them should be a major focus of the advocates of the soliton/wave-AP model to see if these different changes

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between different pairs of phases would all support the waves they suggest should occur. Yet, this seems not to have yet been a focus of study by them. In the main, these good people seem to assume that a L_d/L_o phase transition is just like a liquid crystalline/gel phase transition (Jackson et al. 2020), which is quite an assumption, and seems to be in need of testing. Even if each of these distinct transitions in lipid bilayers of different compositions can support a soliton/wave, then it might be that each transition type produces waves of different amplitudes, durations, speeds, and therefore might this not add some interesting diversity to this phenomenon? Here is a chance for these good people to show how changes in state, found in more complex compositional mixtures, may result in diversity in transition-related phenomena. It should be noted that other systems, aside from lipids, are reported to have multiple phases that they can display often in association with compositional changes (Mathieu et al. 2020; Su et al. 2022; Webber 2022; Zhou et al. 2022). Meanwhile, in biological membranes it might be interesting to consider if the diverse composition of different biological membranes leads to there being new lipid phase states with new properties, perhaps ones not yet discovered via study of simple artificial lipid bilayer systems?

2c. Compositional influences - interference with phase transition cooperativity.

Another interesting outcome with the use of mixtures of lipids and other membrane resident molecules is that different molecules can interact with a given lipid type, and this can alter the ability of that lipid population to engage in the cooperative interactions needed to achieve a phase transition. Thus, again, complex compositions lead to new features emerging which might not be displayed in bilayers made of just one lipid type alone as new combinations of lipids may influence the lipid-lipid interactions that take place (Hac et al. 2005). For instance, Gudmand et al. (2009) note that the addition of a Did-C_{18} marker to a DPPC bilayer shifted the peak of the lipid phase transition to lower temperatures and broadened it. The effect of the addition of new molecules on a lipid's phase transition features is also seen in studies in which two lipid molecules, each capable of engaging in liquid-crystalline to gel phase transitions, are present in various combinations in one bilayer (Fidorra et al. 2009; Heimburg et al. 1992). This effect will next be described in fuller detail.

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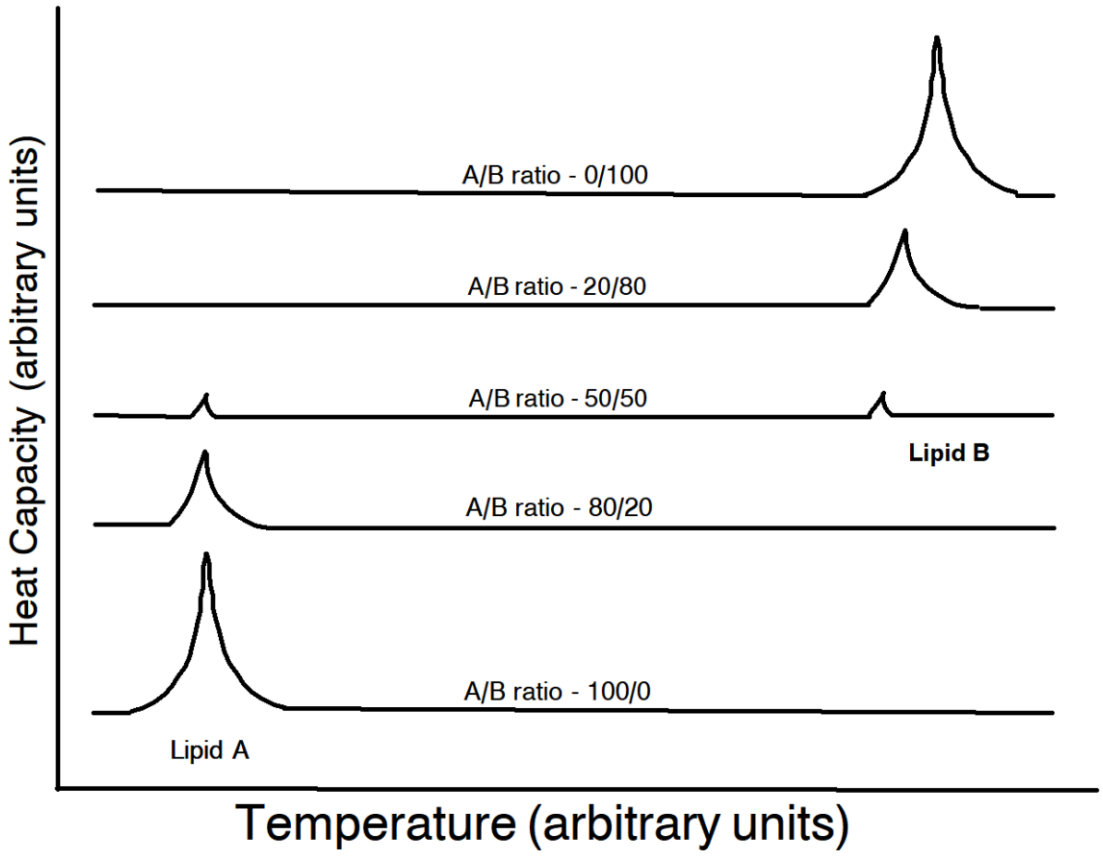

Figure 1. A illustration of the pattern of differential calorimetry data with different mole percent mixtures of two hypothetical phase changing lipids ("A" and "B") which only show a heat capacity peak for the one lipid that is in preponderance and show no heat capacity peak for the lipid that is in the minority. For specific examples of actual data see; Fidorra et al. (2009, Fig. 4) for a study using POPC/pb-cerebroside mixtures, and Heimburg et al. (1992, Fig. 1) for data from bilayers made of mixtures of DMPC/DMG lipids.

As an illustration of this effect, as would be seen via use of differential scanning calorimetry, consider the following hypothetical as given in Fig. 1: Here lipid "A" when in a pure state has a phase transition at a lower temperature, and lipid "B" when in a pure state has one at a higher temperature. With the addition of lipid "B" to lipid "A" the height of the specific heat, Cp, peak associated with the phase transition of lipid "A" is seen to decrease. There are two likely reasons for this decrease. One is that with the addition of lipid "B" there is a lower proportion of lipid "A" present on a mole basis in the system to undergo phase transition, and so the Cp peak observed decreases with lower

 doi:10.20944/preprints202208.0248.v1

molar fraction of lipid "A" is present. If this dilution effect was the sole reason then one might expect that at an "A"/"B" mixture of 50/50 mol% the Cp peak for lipid "A" would be roughly half that seen with pure "A" and when integrated across this peak to get the total heat of transition it should be half that of pure "A," but when put on a per mole basis of lipid "A" should be unaltered. Notice that this is not what was seen in the two studies noted (Heimburg et al. 1992; Fidorra et al. 2009), rather they report, as illustrated in Fig. 1, that in some combinations of lipids once the proportion of "A" in the mixture drops below 50 mol% of the bilayer its phase transition is no longer detectable as no Cp peak is evident. This is often interpreted to mean that at this point every molecule of lipid "A" is no longer interacting just with other "A" molecules, but has significant interactions with lipid "B" instead. This interaction with a different molecular type is thus suggested to interfere with the cooperativity needed for a lipid phase transition to occur. Thus when the mol% of lipid "A" is lower than that of lipid "B" there are no indications in the differential scanning calorimetry scan of a phase transition for lipid "A." Notice also that lipid "B," when present below 50 mol% likewise does not show phase transitions, but once above 50 mol% lipid "B" will have many lipid "B" molecules engaging in predominately interactions with just other lipid "B" molecules and so starts to show a Cp peak as a sign of a lipid phase transition while none is seen at the lower temperature for lipid "A" which is now at too low a mol% to have significant "A"-to-"A" interactions (Fig. 1). This pattern indicates, yet again, that features we might expect to be displayed based on study of pure lipid bilayers may be somewhat altered when two or more lipids are combined in certain ways to make the lipid bilayer. Thus, composition influences the features we actually observe, and this raises the question of whether the complex compositions seen in biological membranes also may produce unexpected influences, which implies that caution may be needed in extrapolating from simple to more compositionally complex systems.

2c(*i*). Protein's influence on lipid cooperativity.

In addition to mixtures of phase-transitioning phospholipids, molecules of other types might be added to a phospholipid bilayer, molecules which do not themselves engage in phase transitions but still may influence the features seen in the bilayer system. One example of biological relevance is the influence of proteins on the lipids' abilities to display a phase transition. Biological membranes are known to have roughly half their mass accounted for by proteins of various types, and these are noted to interact with the surrounding membrane lipids (Heimburg et al. 1993). Therefore it is not surprising that many studies have reported that both peripheral (Heimburg et al. 1999) and integral membrane proteins or peptides (Heimburg et al. 1996; Prenner et al. 1999; Heimburg 2000; Ivanova et al. 2001; Zuckermann et al. 2001; Oliynyk et al. 2007; Ros et al. 2013; Posada et al. 2014) when added to phospholipid bilayer systems can

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alter the properties of the lipid phase transitions seen in the bilayer. Heimburg et al. (1994) note that adding cytochrome c to a DMPG bilayer system, in addition to lowering the ΔCp of the DMPG phase transition, also lowered the changes in viscosity seen in the lipids across its phase transition. In addition, Cañadas et al. (2008) report that the addition of surfactant protein A to rough lipopolysaccharide membranes, with 150 μM $CaCl_2$ present, resulted in the lowering of the ΔCp as observed by differential scanning calorimetry. When a high enough content of protein is achieved it is sometimes reported that the ΔCp peak associated with a normal lipid liquid-crystalline to gel phase transition is lowered in amplitude (Banigan et al. 2018), broadened across a wider temperature range (Heimburg et al. 1994), and in some cases eliminated entirely (Winter et al. 2005). Even the addition of certain amino acids, or phosphocreatine, have been reported to significantly alter liquid-crystalline to gel transitions in phospholipid vesicles (Rudolph et al. 1986; Tokarska-Schlattner et al. 2012). Some proteins have been reported to be able to prevent the rise in permeability that is often associated with lipid phase transitions, presumably by preventing lipid pore formation, and such proteins are suggested to allow some organisms to survive extreme cold (Hays et al. 1996; Tomczak et al. 2002); which seems to imply that some features normally associated with a lipid phase transition, such as lipid pore formation, may be uncoupled from the phase transition and prevented from arising when in association with specific protein types? Xu et al. (2013) report that IgM proteins can influence the dynamics of lipid rafts in the plasma membrane of neurons. Finally, Honigmann et al. (2014) concluded that lipids in the plasma membrane have strong interactions with local proteins, enough perhaps to alter the lipid distribution laterally. Thus, based on these reports, it seems reasonable to suggest that differences in the composition of a lipid bilayer system may be associated with differences in the properties it displays, which seems to run counter to the claims made by the advocates of the soliton/wave model that composition would not significantly alter lipid phase transitions or the resulting waves they suggest to be happening.

There would seem to be good reason, then, to suggest that testing whether or not the soliton/wave-AP model can operate in a lipid bilayer system that contains significant levels of typical membrane proteins, both peripheral and integral, needs to be done? This is especially relevant as the high protein content in biological membranes is noted to alter the enthalpy of lipid phase transitions in living cells Livingstone et al. (1980), and it will be recalled that this enthalpy change, as estimated by integrating across the ΔCp seen, is used in the equations for the soliton/wave-AP model (Table 1). These equations thus imply that if the enthalpy of lipid phase transition is altered the ability to form soliton/waves with certain features may also be altered?

2c(*ii*). Cholesterol influence on lipid cooperativity.

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Another molecular type that is widely reported to be present in many biological membranes, especially in the plasma membrane of neurons, is cholesterol (Welti et al. 1981; Mouritsen et al. 2004; Orth et al. 2012; Lange et al. 2013; Buehler 2016; He et al. 2017), and it is noted to also alter the lipid phase transition and bilayer properties in several ways (Arrais et al. 2007). When low amounts of cholesterol are added to a phospholipid bilayer system, it causes a lowering of the Cp peak seen in differential scanning calorimetry data and broadens the temperature range of the phase transition (Genz et al. 1986; Heimburg et al. 1996), and with higher cholesterol content the detected enthalpy of the phase transition vanishes (Melchior et al. 1976; Almeida et al. 1992; Halstenberg et al. 1998; McMullen et al. 1999; Bhattacharya et al. 2000; Grabitz et al. 2002; Mills et al. 2008; Fidorra et al. 2009; Benjwal et al. 2010; Almeida 2011; Peters et al. 2017; Al-Rekabi et al. 2018). In biological membranes, Bali et al. (2009) report that only when cholesterol was removed from the plasma membrane of platelets was evidence of lipid phase transitions obtained. Similarly, the presence of cholesterol in the plasma membrane of human platelets was reported by Tsvetkova et al. (2000), and the removal of much of this cholesterol was found to alter the conditions under which they could detect a lipid phase transitions by FTIR. This is consistent with the earlier work of Welti et al. (1981) who report that no lipid phase transitions were found in normal plasma membrane, and such phase transitions only appeared after much of the sterol was extracted from the membrane.

Other bilayer properties can also be influenced by the presence of cholesterol. The presence of 40 mol% in a DPPC bilayer is reported to greatly reduce the bilayer's permeability (Mouritsen et al. 2004). Winter et al. (2005) point out that the presence of 30 mol% or more cholesterol in a phospholipid bilayer can suppress the ability of applied pressure to induce a lipid phase transition. The Marsh (1996) article notes that phospholipid bilayers that contain cholesterol have a higher compressibility modulus than do bilayers of pure phospholipids. Jin et al. (2006) notes reports that in lipid bilayers cholesterol can influence the bilayer's dipole potential. Alm et al. (2015) in addition to noting that high cholesterol content can eliminate the lipid gel phase, also report that it can influence the pore inducing abilities of a small peptide added to the bilayer. Al-Rekabi et al. (2018) reports that with higher cholesterol content the stiffness and viscosity of a DPPC bilayer rises. Peters et al. (2017) note that bilayers with a high cholesterol content also removes the changes in volume expansion that normally would be expected to indicate a lipid phase transition, and notes that no phase transition was detected by neutron scattering methods in bilayers with high cholesterol content. Both Ma et al. (2018) and Kashirina et al. (2020) report that the viscosity of regions in the cell membrane can be greatly influenced by the local cholesterol content. The lipid content in biomembranes differs between cell types and membrane types (Lange et al. 2013), but in general the plasma membrane of neurons, and other animal cell types, have a relatively high

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cholesterol content, often in the range of 35-50 mol% of the membrane lipid fraction (Melchior et al. 1976; Livingstone et al. 1980; Jones et al. 1995; He et al. 2017; Amsalem et al. 2018; Lu et al. 2018), and thus only biological membranes with relatively low cholesterol content are viewed as being able to display major lipid phase transitions (Bali et al. 2009). Therefore, the bulk of this work shows that when 40-50 mol% cholesterol is added to a phospholipid bilayer system the differential calorimetry scanning data indicates a loss of any Cp peak, and most workers take this to indicate that there is an absence of any lipid phase transition in such a system. Indeed, Drukarch et al. (2021) make the suggestion that such a high cholesterol content in a biological membrane might account for why, in the view of the soliton/wave-AP model, no APs are found in such cell types as no lipid phase transitions would be expected to occur, which would seem to preclude their happening in neuron plasma membrane?

Once again, it should be recalled that the equations used under the soliton/wave-AP approaches (Table 1) make use of the changes in specific heat capacitance, ΔCp, of the lipid phase transition in their calculation of many of the soliton/wave-AP features. Taken as given, these equations would suggest that if the change in Cp is zero compared to the baseline in the differential scanning calorimetry data then no lipid phase transition occurs. Notice that the lack of a phase transition in a lipid bilayer of certain compositions does not violate these equations, but it would imply that in some contexts the occurrence of soliton/wave phenomena may be unlikely due to the system's composition. One such context that may not show lipid phase transitions, and be unable to display a soliton/wave-AP, may well be the plasma membrane of neurons due to its high cholesterol content? This indicates the need to test carefully whether or not in the plasma membrane of neurons a lipid phase transition is indeed happening during AP firing under normal physiological conditions.

2d. Compositional influences - changes in surface charge densities and potentials.

One interesting feature of the soliton/wave-AP model involves its suggestion that transmembrane voltage changes should occur during the phase transition which is associated with the passage of a soliton/wave. During the lipid phase transition the local volume per lipid molecule changes, and this is argued to alter the local surface charge density which in turn produces a surface electrical potential change on each face of the membrane. A difference between these potential changes on these two faces is then argued to account for the changes we observe as an action potential (Heimburg et al. 2006; Mussel et al. 2019a, 2021). There are two issues we need to explore relative to this model's views on surface potential changes as a means to account for the electrical features of an action potential; one has to do with composition, while the other is more fundamental and has to do with the nature of the surface potential itself.

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So starting with the compositional issue. Obviously, if a flat phospholipid bilayer is used as a model and if it is homogeneous laterally, and symmetrical in terms of having similar surface charge densities on each of its faces, then with the passage of a lipid phase wave each face of the bilayer might be expected to give similar changes in surface potential, which might imply that the surface potential changes would be symmetrical and so cancel each other out and not lead to a net change in the potential across the bilayer. Thus to get a net potential change across the bilayer it seems necessary to assume that there is a surface charge density difference between the two faces of the model bilayer, which implies a need for the two faces to differ in composition. This is the assumption that Heimburg et al. (2006) and Mussel et al. (2019a, 2021) make in their calculations on this matter. Thus, a lipid bilayer that is made of just one lipid type might not be expected to show a net change in the transbilayer electrical field with the passage of a soliton/wave. This seems to imply that the production of net surface potential differences with lipid phase changes is not always assured, but rather is dependent on compositional specifics of the two faces of a bilayer. Indeed, Belosludtsev et al. (2015) notes how changes in the phospholipid composition of vesicles can alter the surface potentials detected. This composition effect might also be seen in the report of Griesbauer et al. (2012) where a pressure pulse applied laterally to a DPPC monolayer produced a surface potential shift of 3 mV, but when the same procedure was applied to a monolayer made from lipids extracted from pig brain, the surface potential shift was just 0.1 mV, implying monolayers of different composition may respond differently when stressed. Bilayers with lipid charge densities of various degrees on one face versus the other might then produce various net electric surface potential changes with soliton/wave passage. Thus to get a soliton/wave passage to create a net local electric field change that would be similar in orientation and magnitude to that of an action potential it may be that only certain lipid compositions and charge density distributions between the two faces of the lipid bilayer are acceptable. This would seem to suggest that certain boundaries of compositional differences between the faces of a membrane would be expected to exist in order for the soliton/wave-AP model to account for the observed electrical features of APs?

This also implies that if a soliton/wave were to pass along a biological membrane, which is heterogenous in composition along its length as many suggest that it is (Brown et al. 1998; Simons et al. 2000; Sahl et al. 2010; Keren 2011; Tsai et al. 2012; Balycheva et al. 2015; Buehler 2016; Gulledge et al. 2016; Rasband et al. 2016; He et al. 2017; Lu et al. 2018; Ma et al. 2018; Mužić et al. 2019; Sankaran et al. 2020; Sengupta et al. 2020; Eisenberg et al. 2021; Galassi et al. 2021), then it might be expected that any net membrane surface potential changes would vary with local composition, thus shifting with location. This might imply that any soliton/wave-AP would be expected to shift in amplitude as it moved along a neuron and encountered local

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compositional differences. Leonenko et al. (2009) notes that even for a simple lung surfactant system the observed surface potentials differ laterally, and especially high cholesterol content can alter the surface potential significantly, showing again that composition changes are related to altered properties. Some cells are noted to alter their lipid composition when under nutritional stress, for instance some bacterial when starved for phosphorous will put non-charged lipids into their plasma membrane that do not contain phosphorous (Sebastián et al. 2016). This might be expected to alter the surface charge density of the plasma membrane, and might then be expected to alter any surface potential changes as well. Indeed, changes in chain length of a phospholipid is noted to significantly alter the change in specific heat capacity seen with lipid phase transition (Schneider et al. 1999), and, given that the equations for the soliton/wave-AP model make direct use of the value of the ΔCp (Table 1), how such changes in membrane lipid content upon changes in growth or environmental conditions, or in nutritional status (Käkelä et al. 2008; Martinière et al. 2011; Lehmann et al. 2020; Vayghan et al. 2020), might then influence the ability of the soliton/wave-AP model to operate would seem to need some consideration?

Another potential issue for the soliton/wave-AP model lies in the pattern of change typically displayed by an action potential. Many APs typically show a shift of membrane potential away from a resting potential that is negative, towards at the AP peak actually having a positive potential (Hodgkin et al. 1939; Huxley 2002a). The modern electrophysiological-AP model accounts for this by noting that the Nernst potential across the membrane for Na^+ is typically positive, and early in many APs the Na^+ current dominates and so the membrane potential swings towards this positive potential. The good people who advocate for the soliton/wave-AP model have not, yet, identified just what compositional/structural differences between the two faces of a lipid bilayer, let alone in a biological membrane, would be required during a lipid phase transition to actually shift the membrane potential from a negative to a positive polarity as seen in APs.

This, again, suggests that the soliton/wave model may have boundaries on its composition if its goal is to account for something like the net electrical field changes that are seen during an action potential produced in a mammalian neuron. It might be possible to approach these matters using lipid bilayer systems of more complex composition than the simple homogeneous bilayers that have been used as the basis for the soliton/wave-AP model. Black lipid membranes, made in the manner suggested by Montal et al. (1972), and as done by Gutsmann et al. (2015), might be produced with different compositions on each face and might then be taken through phase transitions with the net effect of any surface potential changes on the transbilayer potential monitored, for instance. How such changes would then be influenced by composition would be of interest to explore.

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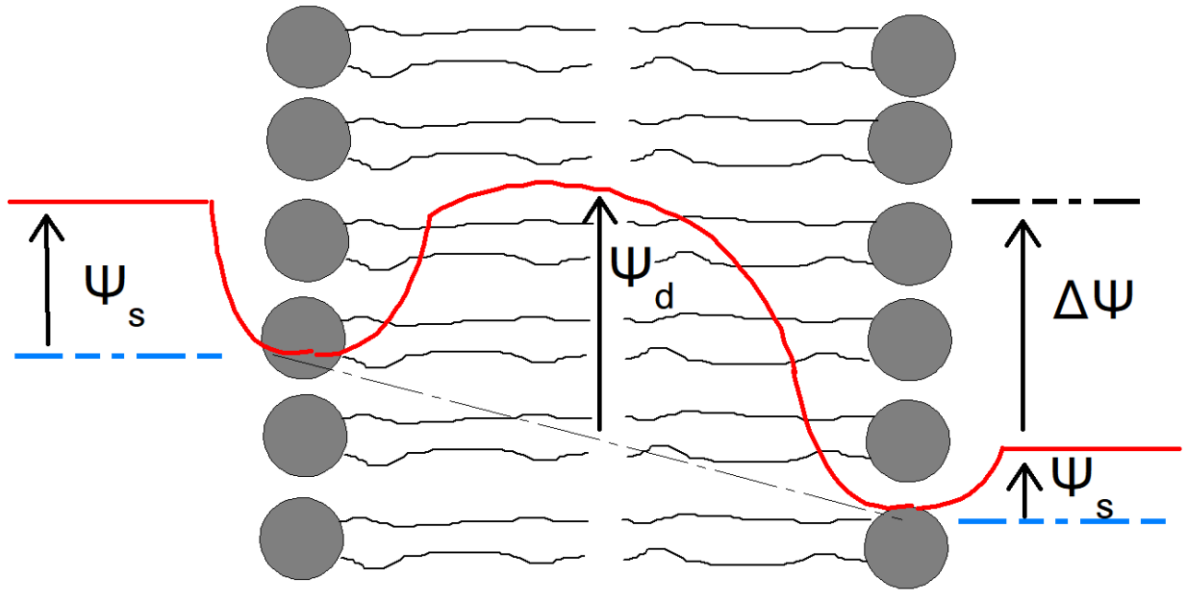

Figure 2. Three types of electrical potentials found in membranes. Of these the transmembrane potential, $\Delta\psi$, is the only one that extends across macroscopic distances. The dipole potential, ψ_d, is found only within the membrane itself. And the surface potential of each face of the membrane, ψ_s, extends only about 1 nm out into the external solutions as charged items and dipole molecules obscure and shield it. Based on the work of Wang (2012) and Galassi et al. (2021).

The second issue relating to the claim by the soliton/wave-AP model that the surface potential changes during a lipid phase transition can account for the action potential has to do with the nature of the surface potential itself, and how it differs from the membrane potential. The membrane potential is a transmembrane potential, and it can be monitored via the use of a pair of microelectrodes, with one in the external solution versus one inserted into the cell's cytosol. Fig. 2 illustrates that the transmembrane potential is a difference between the potential of the two bulk solutions on either side of the membrane and so is a macroscopic potential. This macroscopic nature is indicated by the ability of transmembrane potential changes to be monitored using microelectrodes that are often micrometers away from the plasma membrane across which the membrane potential exists, and also by the use of external electrodes positioned at a considerable distance away

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from the cells that are generating action potentials, as seen in EEG recordings (Cavanagh 2018). In contrast, surface potentials are microscopic potentials (Fig. 2), and their influence is limited to within a nanometer or so of the membrane surface (Wang 2012; Galassi et al. 2021). This limitation is due to both the shielding effect of water, which is a significantly dipolar molecule, and of ions present under normal physiological conditions; with divalent cations, such as calcium ions, having significant shielding effects (Ohshima et al. 1985; Marsh 1996; Oldham 2008; Tsai et al. 2012; Belosludtsev et al. 2015; Lu et al. 2016; Ma et al. 2017b; Galassi et al. 2021; Wieser et al. 2021). Thus whatever surface potential changes might occur during a lipid phase transition, their influence would just be expected to extend out to a distance of under a nanometer in normal solutions. Indeed, Ädelroth et al. (2004) suggests that the influence of charged groups may extend about 7 Å due to ionic screening effects. Of course, such surface potential changes may have significant influences locally. A surface potential may, for instance, induce an outer layer of counterbalancing ions, a sort of double charge layer (Delahay 1996; Buehler 2016; Wieser et al. 2021), forming a layer of local higher ionic concentration, which may influence the activity of some proteins (Yeung et al. 2006). The local nature of this surface potential is seen by the type of methods used to detect it (Chen et al. 2020); one is the atomic force Kelvin probe method (Leonenko et al. 2009; Griesbauer et al. 2012; Tsai et al. 2012; Birkenhauser et al. 2014; Fichtl et al. 2016; Mesquida et al. 2018), or via electrophoresis of vesicles (Belosludtsev et al. 2015), or by the use of fluorescent probes (such as ANS) that are sensitive to surface potential changes (Páez et al. 2013). Thus, unlike the normal transmembrane potential changes seen during an action potential, the surface potentials are microscopic, and often not detectable by microelectrodes which are typically placed too far from the membrane surface to detect them.

Also given that several studies (Leonenko et al. 2009; Mesquida et al. 2018; Galassi et al. 2021) note that proteins can contribute to the membrane surface potential, it may be that changes in protein conformations induced by the AP itself might occur. And thus any changes in surface potential found to be occurring during an AP might be viewed under the electrophysiological-AP model as a secondary feature, and would be local and not contributing to transmembrane potential significantly. In contrast, the soliton/wave-AP model suggests that the magnitude of the surface potential difference changes seen during any presumed phase transition should be similar to that seen during an action potential. Explicit tests of this issue would, of course, be welcome.

Thus the advocates for the soliton/wave-AP model are, once again, making a major claim that would overturn established findings; in this case, that surface potential changes in biomembranes contribute to the macroscopic transmembrane potential and that such changes are detectable by microelectrodes (Heimburg et al. 2006; Fichtl et al.

 doi:10.20944/preprints202208.0248.v1

2016; Mussel et al. 2019a). It might be well if the good people who advocate for the soliton/wave-AP model would produce measurements that support this claim? Perhaps they might show this by using microelectrodes to monitor changes in the transbilayer potential of an asymmetric black lipid bilayer undergoing a phase transition with the presence of screening ions at normal physiological concentrations? Antonov et al. (2003) in a study of the changes in lipid bilayer capacitance during temperature induced changes in lipid phase did not report any shift in transbilayer potential in association with the lipid phase changes. The advocates for the soliton/wave-AP model might wish to repeat such work, and see if they can confirm that transbilayer potentials of the magnitude seen in an action potential can indeed be observed with lipid phase changes in asymmetric lipid bilayers and explore what compositional differences between the bilayer faces would be needed?

We might also get at this issue of whether or not the surface potentials contribute to the transmembrane potential by examining its influences in a living cell. Eisenberg et al. (2021) note that the plasma membrane of cultured mammalian cells typically has anionic carbohydrates on its outer face, and reports that treatment with neuroaminidase removes these charged groups and so alters the surface potential, and this approach for altering the surface charges on one membrane face is also suggested by de Lichtervelde et al. (2020). Under the soliton/wave-AP model would such a neuroaminidase treatment to the outer face of the plasma membrane of a cultured neuron cell then lead to an alteration in the features of the AP seen? Also, recently the advocates of the soliton/wave-AP model have reported that the cooling of cultured neuron cells, to well below the normal physiological range, yielded data that seem consistent with a lipid phase transition (Fedosejevs et al. 2022). The soliton/wave-AP model would predict that above the phase transition temperature the plasma membrane would be in one phase and so display one transmembrane potential, but then by cooling the cell to below that transition temperature the lipids of its plasma membrane should shift into another lipid phase with a different set of surface charge density differences between its two membrane faces. If the soliton/wave-AP model is correct, then this change in surface charge densities with changes in lipid phases should result in a detectable change in the magnitude of the plasma membrane potential, and should be expected to be similar in magnitude to the swings in membrane potential changes seen during an action potential. Holding the cultured neuron cell at a warmer temperature would thus be expected to produce one transmembrane potential, close to the normal resting potential, while cooling the cell down below the temperature at which the lipid phase transition occurs would then be expected to move the transmembrane potential closer to the peak seen during an AP, and keeping the cell cool would then be expected to hold it at this new transmembrane potential. The advocates for the soliton/wave-AP model may wish in this way to test

 doi:10.20944/preprints202208.0248.v1

their assertion that differences in changes in surface potentials account for changes in the transmembrane potential in living cells?

2e. Summary: Implications of compositional influences on phase transitions for the soliton AP model.

The arguments presented above indicate that once we move away from pure homogenous lipid bilayers made up of just one lipid type that things can get very complicated indeed. Conditions that in a homogenous lipid bilayer would induce a lipid phase transition, might fail to induce a phase transition when a mixture of lipids are present in the bilayer, or when proteins are added. Also in mixtures the formation of new types of lipid phases arises, which implies new phase transitions between various pairs of lipid phases might be possible. The implication of these data from studies of phospholipid bilayers of various compositions for the application of the soliton/wave model to biological membranes is that a lipid phase transition of a pure homogenous bilayer *in vitro* may not indicate whether or not such a transition occurs in a biological membrane *in vivo*. This is due to the presence of other molecular types in the biological membrane that might interfere with the intraspecific molecular cooperativity needed for a lipid phase transition, and so the lack of a phase transition might indicate that there would be no soliton/wave related phenomena in certain biological membranes. Thus while there is good evidence of certain types of simple phospholipid bilayers being able to display lipid phase transitions, and so exhibit soliton/waves and their associated phenomena, there is also good reason to question if this truly happens in the mixtures of molecular types found in many biological membranes. Indeed, while recognizing the use of lipid bilayer and monolayer systems in our studies of membranes, Feigenson (2007) suggests that it is inappropriate to assume that the features found in such simple systems translate directly to biological membranes. Thus, it would be of interest to create lipid bilayers that more closely mimic the more complex composition of biological membranes. This might allow tests of whether any lipid phase transitions occur, while noting the differences in phase transition characteristics that might be displayed when transitioning between various pairs of lipid phases. The range of changes, while still within physiological conditions, that can induce phase transitions might then be identified. Therefore, without considering the composition of the membrane to be modeled, it might be inappropriate to conclude from the results so far obtained using pure homogeneous phospholipid bilayer systems just what will happen in a biological membrane. Thus composition may matter greatly in these cases, and the claims made by the advocates of the soliton/wave-AP model that its equations apply to all bilayers and membranes universally no matter their composition are still open to questioning.

3. On several claims of lipid phase transitions in biological membranes.

 doi:10.20944/preprints202208.0248.v1

The soliton/wave-AP model makes the assumption that in biological membranes something like a liquid-crystalline to gel phase transition occurs, and so a soliton/wave based on a lipid phase transition happens. This assumption has been questioned as it seems to fly in the face of many reports in the literature which imply that lipid phase transitions are damaging to cells and so are kept rare (Meissner 2018). There are several recent reports that claim that such lipid phase changes occur in the plasma membrane of excitable cells. Fedosejevs et al. (2022) presents some credible evidence of detecting a lipid phase change in a neuron, but its association with action potentials is not yet clearly established. Mussel et al. (2021) in their review suggest that the work presented by Shrivastava et al. (2020) in a preprint (which considers selected evidence presented in Lee et al. 2017) as being consistent with a lipid phase transition in the neuron plasma membrane in association with an action potential. And, both Heimburg (2021) and Jackson et al. (2020) cite Mužić et al. (2019) as presenting evidence for lipid phase transitions occurring in biological membranes. As these claims are quite relevant to the soliton/wave-AP model as an explanation for the action potential in neurons, it is appropriate to next consider this evidence. In this section, the focus will be mainly on the Mužić et al. (2019) study in terms of some technical issues that might have strengthened it, as well as how its findings might be viewed relative to physiological conditions. Some of the other claims about lipid phase transitions occurring in biological membranes will then be touched upon in section 4.

3a. Claims of lipid phase transitions - Methodological issues.

Mužić et al. (2019) used extracts from nervous tissue of several species as their sample material. One issue that arises when isolating any sample from a biological source is that of degradation during the isolation process. There are various enzymes, lipases and proteases, that can be activated during extraction. So a good practice is to include from the start of the isolation process known labeled molecules, either lipid or protein, which can then be checked at the end of the process to see if degradation is a major issue. Prenner et al. (1999) also note that the passage of such an extract through the range of temperatures typical of a differential calorimetry scan can result in the cleavage of some types of lipids, which may then alter the phase transition features of a sample. However, Mužić et al. (2019) made no use of these sorts of controls to check for degradation during isolation or measurements. Therefore, we have no way of knowing if the sample they obtained was intact, degraded, if there were lipases and/or proteases still present and active when they took their measurements, or if the carrying out of calorimetry induced changes in the lipids and so in their properties. If enzymatic degradation of the sample was ongoing, then the data collected from the sample might be expected to shift over time leaving open the question of whether what was observed represents the conditions found

 doi:10.20944/preprints202208.0248.v1

in the original source or if it reflects the influence of degradation products created during isolation of the sample and so are artifacts of isolation or of measurement. Thus the use of added known lipid and protein markers as controls to check for potential degradation would have been helpful.

In terms of the material used, Mužić et al. (2019, pg. 6) state: "We have chosen central brain and spinal cord tissues because we assume that in these tissues most membranes are involved in signal conduction. However, we cannot distinguish different membranes. Our results represent an average over all types of membranes in the tissue." A simple homogenization was thus used, and this included material from non-excitable as well as excitable cells. And this approach also lumped together material from the plasma membrane with all the internal membranes of the cell, including the endoplasmic reticulum and others, as well as all the cells' soluble components such as proteins and ribosomes. Clearly, it would have been better if the crude homogenate had been fractionated so that a purified plasma membrane fraction could have been obtained. Such a process of fractionation typically involves identifying the plasma membrane fraction by the use of enzymatic markers, and the checking of enzymatic markers from other membranes of the cell so that contamination of the plasma membrane fraction by endoplasmic reticulum, mitochondria, lysosomes, etc., can then be done to assess purification, as done in the work by Livingstone et al. (1980) and by Roy et al. (1997). The use by Mužić et al. (2019) of a unpurifed homogenate leaves open the question whether any findings they obtain can be attributed to just the plasma membrane of excitable cells. Thus as Mužić et al. (2019, pg. 9) themselves state: "An important caveat in the interpretations of our data is that they are not obtained from clean membrane preparations." Thus it is not clear if their results should be attributed to a specific cell type, or to a specific membrane type, or even to certain soluble factors found in their homogenate. This is critical, as lipid bilayer systems, and so by extension plasma membrane fractions, with a high cholesterol content might not show a clear phase transition via differential scanning calorimetry (Peters et al. 2017), while samples derived from the endoplasmic reticulum are reported to be lower in cholesterol (Mouritsen et al. 2004) and so might be producing much of the differential scanning calorimetry (DSC) signal that Mužić et al. (2019) report. This ambiguity as to which membrane may account for the DSC signal might have been largely avoided if neuronal cells of a specific cell type had been grown in culture to a stage where they could display action potentials, and then harvested and the plasma membrane fraction purified from them for study. Otherwise the use of such a broad homogenate by Mužić et al. (2019) seems to preclude definite conclusions about specific source(s) of any data obtained, and so makes much more difficult the addressing of the central question of whether or not in the plasma membrane of excitable cells there is any lipid phase transition occurring under physiological conditions in association with the action potential firing.

 doi:10.20944/preprints202208.0248.v1

Thus with a crude homogenate as their starting material, Mužić et al. (2019) then proceed to apply the method of differential scanning calorimetry (O'Neill 1966; Heimburg 2007; Haynie 2008), and attempt to discern if across the conditions they apply there is evidence of any sort of lipid phase transition. This was done by noting the presence of peaks in the specific heat capacity (Cp) that may occur across a scan relative to a designated baseline [for a summary of how setting such a baseline is typically done see the supplemental material for Mužić et al. (2019), as well as the article by Ivanova et al. (2001)]. However, differential scanning calorimetry in addition to being used to examine lipid phase transitions can also be used to assess changes in protein conformations and even changes in folding of nucleic acids as exist in isolated ribosomes (Heus et al. 1983; Gluick et al. 1994; Shnyrov et al. 1997; Krupakar et al. 1999; Bonincontro et al. 2001; Lee et al. 2002; Nguyen et al. 2006; Gill et al. 2010). It should be recalled that protein conformational changes are an essential part of the modern electrophysiological AP-model, and so voltage sensitive domains in proteins shift their positions due to stress placed on them with changes in the transmembrane potential. Such protein conformational changes largely involve intramolecular interactions, and so may be argued not to represent phase transitions. Phase transitions may be argued instead be due to altered patterns of intermolecular interactions happening in the bulk solvent (*i.e.*, between water molecules, or between lipids in a bilayer or membrane). This distinction matters in terms of how much cooperativity might occur. In this regard it should be noted that calorimetry studies of protein conformational changes have reported that some of their changes can be irreversible, and so characterized as causing the irreversible denaturation of the protein, but it is also reported that many proteins show reversible conformational changes across several calorimetric scans (Akiyama et al. 2020).

Thus a major issue in the work of Mužić et al. (2019) is how to characterize any specific heat capacity peak their calorimetry scan detects in terms of whether it is due to lipid phase transitions or changes in other molecules, and they attempt to do this in two ways. First, Mužić et al. (2019; pg. 2) claim that the pressure treatments applied while doing differential scanning calorimetry can be used to distinguish which Cp peaks are from lipid phase changes and which are from protein conformational changes. This is a potentially significant issue as it has previously been reported that protein conformational changes can have detectable enthalpy changes (Heimburg et al. 1991), so both lipids and proteins can contribute significantly to differential scanning calorimetry data. Indeed, Grabitz et al. (2002, pg. 308) state: "A further complication is that, in calorimetric experiments, it is difficult or even impossible to distinguish heat-capacity events originating from lipids and from proteins." So to support their claim of being able to distinguish via pressure treatments which differential scanning calorimetry peaks are

 doi:10.20944/preprints202208.0248.v1

from which molecular types Mužić et al. (2019) cite the previous work by Ebel et al. (2001). A problem here is that Ebel et al. (2001) did not examine protein calorimetric responses to pressure treatments at all, but rather only examined the calorimetry of lipid phase transitions. Elsewhere, Mužić et al. (2019, pg. 3) cites work by Royer (2002) in support of this claim that the transition temperature of proteins and lipids will be shifted in different directions when under pressure. But Royer (2002) relates pressure treatments on proteins to their volume changes which they note to vary in direction depending on the specific protein examine, and did not specifically report the nature of any shift in a calorimetric Cp peak due to a pressure treatment that would occur during a differential scanning calorimetry examination of proteins. So it seems that Mužić et al. (2019) are implying that certain specific heat peaks can be attributed to proteins and others to lipids by their sensitivity to pressure, and perhaps such a difference in sensitivity does exist, but they offer no direct findings in support for this claim. And, given that Franco et al. (2012) note that the human lactoferrin protein's conformation is rather stable up to pressures of about 400 MPa (about 4000 bars), not all proteins may respond equally to pressure treatments?

The second way in which Mužić et al. (2019) attempt to discern which Cp peak is due to lipids is by claiming that running a differential calorimetry scan to relatively high temperatures would irreversibly denature the proteins, and so removes their contribution to subsequent calorimetric scans so that what is left is assumed to be due to changes in lipid phases. This suggestion that any protein conformational changes would be irreversible while lipid phase changes would be reversible is also made by Fedosejevs et al. (2022). It is true that high heat can irreversibly denature some types of proteins, but that does not always imply that the proteins then become calorimetrically inert. Indeed in attempting to use differential scanning calorimetry to detect lipid phase transitions in isolated red blood cell samples Grabitz et al. (2002) found that the heat signal from the proteins, even when denatured, made it impossible to detect any heat signal from a lipid phase transition. Thus denaturating proteins does not make them calorimetrically inert in all cases. So Mužić et al. (2019) are assuming that the high temperature conditions applied in the first differential calorimetric scan would irreversibly denature all proteins and so remove their contribution in subsequent scans. That assumption seems to be in need of testing as Privalov et al. (2000) notes that there are proteins that can be reversibly denatured across broad ranges of temperatures. Indeed, Melchior et al. (1976) also report such an occurrence with proteins reannealing after high heat exposure, and so such proteins would then be expected to contribute heat of conformational changes in each of the calorimetry runs undertaken. The work of van Osdol et al. (1991) shows that cytochrome c has highly reversible folding/unfolding conversions. A review by Mazurenko et al. (2017) on the study of proteins by microcalorimetric methods notes that many other proteins have fully reversible changes in conformation after exposure to heat.

 doi:10.20944/preprints202208.0248.v1

Jayaraman et al. (2011) report that human apoA-1 in free solution does show reversible protein unfolding across cycles of calorimetry heating and cooling. Also relevant is the work of Kaletunç et al. (2004) who report that when whole bacterial cells are passed through differential scanning calorimetry analysis specific heat capacity peaks can be attributed to DNA and to ribosomes, without any attribution to lipid phase changes at all. Several studies (Lee et al. 2002; Nguyen et al. 2006) which take whole bacterial cells through differential scanning calorimetry suggest that major heat capacity peaks could be attributable to ribosomal conformational changes. This leaves the claim by Mužić et al. (2019) that with a crude homogenate as source material they can discern which peak(s) in specific heat capacity are associated with lipid phase changes in some doubt, as the alternative hypothesis that some of these peaks might be due to reversible protein or DNA or RNA conformational changes seems to have not been fully examined. If instead they had started with a well purified plasma membrane sample some of these and other reservations might have been addressed.

3b. Claims of lipid phase transition - findings.

In any event, Mužić et al. (2019) go on to report the conditions they found that can result in a pattern of calorimetry data which they argue indicate lipid phase transitions in their extracts from nervous tissue. These conditions include: Lowering the temperature by 10-20°C below body temperature, application of from 100-196 bars of pressure above normal, altering the external pH by 4 units, and altering the external osmolarity by the addition of 300 mM NaCl (Mužić et al. 2019). These claims are echoed by Fillafer et al. (2021) who suggest that a cooling by 15-35°C, or application of about 810 bars of pressure, or a drop in external pH by 1-3 units would induce a lipid phase transition, and so this is suggested as a means for the initiation of the soliton/wave-AP. What follows next is an attempt to place each of these findings within a physiological context as a means to assess whether these conditions can occur *in vivo*.

3c. Claims of lipid phase transition - Physiological Temperature range.

The finding by Mužić et al. (2019) that lowering the temperature by 10-20°C below the typical body temperature could induce what might be a lipid phase transition in their crude extracts may be consistent with a report by Fedosejevs et al. (2022) which found that a cultured neuron cell may show signs of a possible lipid phase transition when cooled from a normal 37°C down to about 17°C. However, it must be noted that in humans the normal healthy temperature range is roughly 36-40°C (Reece 2015; Beker et al. 2018). Excursions of temperature changes significantly beyond this range have been noted to lead to many potentially life threatening conditions (Lim et al. 2008). In any event, just how sections of the plasma membrane of a neuron *in vivo* would have its temperature

 doi:10.20944/preprints202208.0248.v1

altered to this extent in the first place so as to induce the presumed lipid phase transition needed by the soliton/wave-AP model has not been described. In the absence of any major finding of microbursts of cooling occurring just before each and every action potential firing in normal nervous tissue, it would seem unlikely that temperature drops of 20°C are plausible under normal physiological conditions. This objection would, of course, be removed if solid evidence for lipid phase changes was forthcoming from human neurons in their normal temperature range of 36-40°C, but no such evidence has been presented yet by the advocates of the soliton/wave-AP model. The matter is made even more confused by the claim of Heimburg (2019, pg. 39) that changes in protein and nucleic acid conformations are said to be happening at temperatures "... close to physiological conditions..." but here Heimburg notes that for DNA the temperature at which this happens is 65°C or more! Thus exactly what is meant by the advocates of the soliton/wave-AP model in their references to temperatures said to be "close" to physiological conditions seems to need some clarification.

It might also be noted that Greffrath et al. (2009) reports that exposure of some neurons to 42-47°C for a few seconds can induce AP firing. Paajanen et al. (2004) report that heat stress can lead to changes in ion channel activity, and so alter AP firing features in fish. Also a report by Fribrance et al. (2016) modeled this heat induced AP firing through a modification of the original Hodgkin and Huxley equations (Hodgkin et al. 1952a) to take heat induced changes in membrane capacitance into account. The electrophysiological-AP model largely accounts for the ability of some types of neurons to continue to fire APs at various temperatures by noting the changes in the types of alpha subunit used in the voltage-sensitive Na^+ channels (Touska et al. 2018). Thus, if the lipid phase transition that the soliton/wave-AP model requires for its operation is meant to be associated with cooling, then how does this model account for the induction of AP firing upon exposure to heat?

Furthermore, the suggestion is made by Schneider (2021), and by Kang et al. (2020), that at temperatures below this presumed lipid phase change no action potential firing would be possible because, under the soliton/wave-AP model, at such low temperatures the lipid phase would be unlikely to be further altered and so no soliton/wave-AP could be generated. This claim seems to run counter to a number of reports of action potentials being observed at temperatures much lower than 17°C. For instance, Spyropoulos (1957, 1964) reports action potentials in squid giant axons and in neurons of other animal species down to 4°C. Abbott et al. (1958) reports detecting APs in nerves of crab at temperatures down to 0°C. Both Keynes et al. (1965) and Howarth et al. (1968) working with nerves from rabbit could detect APs at temperatures down to 5°C, a finding that Ritchie (1973) confirmed later. Franz et al. (1968) reports action potentials in isolated cat neurons when taken down to 7.2°C before any sort of cold block was detected. In a study of the flux of

 doi:10.20944/preprints202208.0248.v1

$^{42}K^+$ associated with action potential firing in garfish nerve fibers Ritchie et al. (1975) were able to see action potential firings down to 0℃. While in mammalian excitable cells Ritchie et al. (1956) had noted that cooling down to 10℃ caused no sudden block in AP firing, rather a gradual change in some AP properties in step with gradual cooling was noted. In contrast, at the other end of the temperature range, both Money et al. (2009), working with neurons in locust, and Klumpp et al. (1980) working with cat nerves noted that heat treatments above 40-45℃, if applied long enough, can inhibit action potential firing; they presume this would be due to protein denaturation induced by the heat and note that this heat block was often irreversible. Thus the advocates for the soliton/wave-AP model might wish to repeat the study done by Fedosejevs et al. (2022) and test to see if indeed, in a cultured mammalian neuron which shows action potentials at normal physiological temperatures, no AP firing occurs at temperatures below the presumed lipid phase transition temperature of 17℃? If action potentials in such mammalian neurons are seen below this presumed lipid phase transition temperature that would seem to challenge their soliton/wave-AP model. This is a test of their model that seems not yet to have been made by its advocates.

In contrast, electrophysiological theory indicates that changes in temperature will alter the resting membrane potential. This can be seen, for instance, in the temperature term that is present in the Goldman-Hodgkin-Katz equation (see eq. 2), and the permeability terms in it which reflect the actions of the protein-based ion channels may also change with temperature (Lei et al. 2019). The study by Bolton et al. (1981) found that lowering the temperature of a human arm over a physiological range did alter the compound action potentials observed in a gradual manner, which would seem to be consistent with the electrophysiological-AP model. Of course, sudden changes in protein-based ion channel activity can occur in those specific channels that are reported to operate in the sensing of temperature (Brauchi et al. 2004; Laursen et al. 2015), but that is a separate matter from the temperature influences broadly on something like an AP. Thus the advocates for the soliton/wave-AP model suggest a temperature cut off below which APs would be proposed not to occur, but does not seem to account for the gradual change in AP properties seen with temperature changes across the normal physiological range?

3d. Claims of lipid phase transition - Physiological pH range.

As noted previously, Mužić et al. (2019) also reports that a change in pH in the range of 4 pH units may induce extracts from nervous tissue to show what is presumed to be a lipid phase transition. This suggestion is echoed by the Fillafer et al. (2016) article which suggests that external acidification of a *Chara* sp. cell might stimulate action potential firing. Also in the Fillafer et al. (2021, 2022) articles there is the suggestion that lowering

 doi:10.20944/preprints202208.0248.v1

the external pH near a synapse by 1-3 pH units, down to below pH 5.6, might induce a flux of protons across the plasma membrane which may then induce a change in the membrane potential which in turn, they suggest, would induce a lipid phase transition in the neuron, and so initiate a soliton/wave-AP. This hypothesis needs to be tested. But this seems to be an alteration of the soliton/wave-AP model which previously did not consider any flow of ions as being involved in AP induction as ion flow and ion channels were thought not to play a role in APs (Heimburg et al. 2006; Heimburg 2010; Vargas et al. 2011; Appali et al. 2012; Mosgaard et al. 2013; Gonzalez-Perez et al. 2016)? The Fedosejevs et al. (2022) article also suggests that an external acidification by 2 pH units may induce some sort of lipid phase change in cultured neuronal cells, but they have not shown that such a shift results in a membrane potential shift of the magnitude that would be expected during an action potential. Be that as it may, the suggestion that a change in external pH in the 1-4 pH unit range as a means to induce a lipid phase transition, and so initiate a soliton/wave-AP in the cell, would seem to depend on the assumption that such changes in pH actually occur *in vivo*. Thus a review of reports in the literature to see to what extent pH can vary under normal physiological conditions will next be presented.

Generally the cell cytosol of many mammalian cells is held in a pH range of 6.7-7.7, with cells that shift their metabolic rates often showing the more extreme shifts within this range (Crampin et al. 2006; Hayashi et al. 2008; Morgan et al. 2009; Counillon et al. 2016; Rajendran et al. 2018). In terms of the extracellular pH, Chesler (2003) suggest that in the mammalian nervous tissue the external pH is often limited to changes of under 0.2 pH units. Chen et al. (1991) in examining the influence of the release of the neurotransmitter GABA on the local extracellular pH report an alkalinization in the range of 0.02-0.12 pH units. Fliegel (2019) describes the role of a Na^+/H^+ exchange system in the plasma membrane that is used by some cells to help avoid acidosis of the cells. And Theparambil et al. (2020) describe the use of a bicarbonate transport system by astrocytes in the nervous tissue to limit changes in extracellular pH to under 0.1 pH units, and note that loss of this buffering system can lead to various sorts of mental disorders. Several other reports note that extracellular acidification can be damaging: Foster et al. (2021), Jang et al. (2020), and Sivils et al. (2022) all report that an external pH of 6.5 or less in nervous tissue is often associated with acidosis-related neuropathologies and neuronal damage. Jones et al. (2011) found that an external pH of 6.0 led to inhibition of the cardiac voltage gated sodium ion channel, and that such a pH drop was seen in association with cardiac arrhythmia. While Toyoda et al. (2008) report that an external pH of 6.3 could inhibit the activity of a K^+ ion channel in cholinergic neurons. Huang et al. (1999) has reported that several neurotransmitter receptors and ion channels of neurons are inhibited by extracellular acidification, with acidification of 0.5 pH units being associated with seizures, and drops of 1 pH unit being associated with permanent cellular damage. These studies seem to suggest that both internal and extracellular pH are under rather

 doi:10.20944/preprints202208.0248.v1

tight regulation in mammalian excitable tissue. Thus any suggestion of changes in extracellular pH of over 0.5 pH units may need justification?

In other work by the advocates of the soliton/wave system, Fillafer et al. (2016, 2022) suggest that acidification of the external medium might enhance the rate of action potential firing in nervous tissue. However, the opposite seems to be reported in some cases with external acidification often inhibiting neuron action potential firing (Chesler 2003). For instance Jang et al. (2020) reports that an extracellular acidification by 0.5 pH units decreased activity at an excitatory synapse. Hille (1984) notes that an external acidification by 2 pH units inhibits action potentials in some cases, and refers to this as an "acid block" to action potential firing. And it might also be noted that Hille (1968) and Drouin et al. (1969) report that with external acidification the conductances of both the Na^+ and K^+ currents associated with action potentials are lowered, suggesting that their associated channel activities may be pH sensitive. While the report by Soto et al. (2018) suggests that pH shifts just within the synaptic cleft might be significant, and might activate acid sensing H^+ channels in post-synaptic membranes, they suggest that any acidification of this type would act through ion channel activation and do not suggest any lipid phase changes being involved. Thus the suggestion by Fillafer et al. (2016, 2022) that external acidification might induce the lipid phase transition needed for the soliton/wave-AP to operate in neurons, seems to be contradicted by the reports of Hille (1968, 1984), of Drouin et al. (1969), and others which suggest that such acidification suppresses the activity of the ion specific channels and thus inhibit AP firings.

Thus if the soliton/wave-AP model requires changes in external pH of over a few tenths of a pH unit it might fall outside of the normal physiological range? To counter this objection, the advocates of the soliton/wave-AP model would need to produce evidence that at specific locations along healthy neurons in the mammalian central nervous system there are pH drops in the range they are suggesting, and which are closely associated in time with lipid phase changes and actual action potential firings. Without such actual evidence of operation *in vivo*, this pH effect may be yet another of many such effects that we can induce *in vitro* (*i.e.*, like electroporation); a real phenomenon perhaps that cells can be forced to display, but which is not actually used by life under normal conditions?

3e. Claims of lipid phase transition - Physiological pressure range.

Finally, Mužić et al. (2019) reports that the application of 100-196 bars of pressure to the crude homogenate they had from the central nervous system of pigs can induce what is thought to be a lipid phase transition as detected by a peak in specific heat capacity during differential scanning calorimetry. This may be consistent with the findings of

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Matsuki et al. (2019) who reported that treatment of phospholipid bilayers with from 220-3000 bars of pressure could alter their phase state. This suggestion that lipid phase changes in biomembranes might be induced by pressure is also made by Fillafer et al. (2021). Thus they imply that pressure changes might be one way a cell might induce the lipid phase transition needed for their soliton/wave-AP model to operate in a living cell.

It has been reported that during an action potential there is a change in cellular internal pressure, but it is very small, in the 0.1 millibar range. However in addition to being observed in association with an action potential such a small pressure shift can also be brought about by a membrane hyperpolarization which does not lead to any action potential firing (Terakawa 1985). Both Shimmen (1997) and Staves et al. (1993) examined how application of pressure, in the range of about 1 millibar, to a small area of the plasma membrane of the internodal cell of *Chara* sp. could induce action potential firing. Furthermore, Julian et al. (1962) notes that the such local pressure responses seem to be associated with changes in the conductance of the membrane, and are dependent on the presence of certain extracellular ions, which in the modern context might imply an involvement of touch-sensitive ion channels? In terms of local force that can induce responses Muhamed et al. (2017) notes that application of force in the piconewton range to cell surfaces can often initiate mechanotransductive effects, so any sort of local pressure effects that might be proposed would likely have to operate in this range of forces. In terms of lipid phase transitions, the Winter et al. (2005) article in looking at lipid bilayer responses to pressure found that both cholesterol and protein content can reduce or eliminate pressure induced lipid phase changes, and even with a pure DPPC bilayer the pressure change needed to induce its shift from liquid-crystalline to gel phase was found to be over 300 bars of pressure, which is roughly the pressure that is seen in the ocean at a depth of several thousand meters! It should be noted that Spyropoulos (1957) working with the squid giant axon found that upon application of roughly 200-480 bars of pressure to the axon this did lead to stimulation of spontaneous AP firings. But if hundreds of bars of pressure are needed to induce lipid phase transitions, as Mužić et al. (2019) and others suggest, while action potentials are found by others to be inducible by local application of just millibars of pressure, then the suggestion that lipid phase transitions are involved here would seem to be brought into some doubt as the extent of pressure needed to induce lipid phase changes seems to be orders of magnitude greater than the light pressures reported to be able to induce action potentials in some cases?

In terms of what sort of pressures can be tolerated in the central nervous system both Harary et al. (2018) and Canac et al. (2020) suggest that a rise in intracranial pressure by just 25 mm Hg (*i.e.*, about 0.03 bar) is often found to be associated with severe headaches, neurological disorders, and even damage to brain tissues. Thus it might be implied that pressure shifts of even one bar in the central nervous tissue could be out of the bounds of

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normal physiological range, and start to enter what happens with impact related pressure spikes leading to brain damage. And yet the advocates for the soliton/wave-AP model are suggesting that hundreds of bars of pressure might be brought to bear in the nervous tissue regularly. Evidence of the occurrence of such pressure spikes in normal healthy nervous tissue is clearly needed to justify their claims.

3f. Summary: About limits imposed by physiological ranges.

When a phenomenon such as a lipid phase transition is found to occur only under conditions that are outside of normal physiological ranges, that might suggest that the phenomenon, while inducible by applying extreme conditions, may not normally occur *in vivo* under normal physiological conditions. Such would seem to be the case for the induction of lipid phase transitions in the plasma membranes of excitable cells. As noted above, the conditions of pH, temperature, and pressure which the advocates of the soliton/wave-AP model suggest are needed to induce such lipid phase transitions seem to be well outside of normal physiological ranges. This perhaps makes the lipid phase transitions seen in these cells seem somewhat similar to the phenomena of electroporation (Joersbo et al. 1990; Pakhomova et al. 2014), or even similar to atomic nuclear fusion (Post 1976), each of which can be caused to be induced but the needed conditions are so outside of normal physiological ranges as to preclude our concluding that life makes use of such phenomena on a regular basis. Also this apparent exclusion of a regular use of lipid phase transitions by life may be seen to be consistent with the view, as presented previously (Meissner 2018), that due to both their damaging nature (Welti et al. 1981; van Bilsen et al. 1994; Oldenhof et al. 2013), and how hard they are to induce, such lipid phase transitions are maladaptive and so may not normally be seen to happen in nervous tissue?

However, the advocates for the soliton/wave-AP model reach a different conclusion. Mužić et al. (2019, pg. 1) states: "Since the feature of a transition slightly below physiological temperature is conserved even when growth conditions change, we conclude that the transitions are likely to be of major biological importance for the survival and the function of the cell." And goes on to state (Mužić et al. 2019, pg. 8): "Thus, the physiological temperature in all of the preparations was found to lie just between the lipid transition and the protein unfolding transitions such that minor perturbations of the membranes will move the membranes into the transition regime." The critical issue here would seem to be what exactly is meant by being "slightly below" normal conditions and what are "minor perturbations"? By this do they mean to suggest a 10-20℃ cooling, or an acidification by several pH units, or a change in pressure of several hundreds of bars, are "minor" changes, even when such changes are potentially lethal? By this sort of reasoning, one might then have to conclude that the ability to electroporate a cell

 doi:10.20944/preprints202208.0248.v1

membrane, being seen across a wide range of species, must also then be critical in some way for cell viability or function? Thus just because we can induce something artificially does not make it adaptive *in vivo*. Yet the advocates for the soliton/wave-AP model seem willing to make such a leap of faith.

The soliton/wave-AP model has the implicit assumption that such lipid phase transitions are very common and used in an adaptive manner in biological membranes. However, the temperature, pH, and pressure changes that are proposed as being needed to induce such a lipid phase transition (Mužić et al. 2019; Fillafer et al. 2021) seem to be outside of the normal physiological ranges for these items. Thus to demonstrate that such lipid phase changes do indeed occur, the good people who advocate for the soliton/wave-AP model would then need either to show that their model operates within these physiological ranges in normal living neurons, or would need to provide new data demonstrating that the conditions they report as being needed to induce lipid phase changes in extracts do actually occur normally *in vivo* in nervous tissue. Without such new information the conditions they suggest as being needed for the induction of lipid phase changes seem to be so far outside of the normal ranges of pH, temperature and of pressure so as to make the induction of lipid phase changes under normal conditions to seem to be rather doubtful.

One oddity with regard to the conditions explored by the advocates for the soliton/wave-AP model for the induction of lipid phase transitions has been a relative lack of consideration of the influence of membrane potential depolarization. It is well known that membrane potential changes of a few tens of millivolts are able to induce AP initiation in excitable cells (Hille 1984; Golod et al. 1998; Henze et al. 2001). So if these APs are, as the soliton/wave-AP model suggests, due to a lipid phase transition, then it seems necessary for the advocates of this soliton/wave model to show that a similar depolarization of the plasma membrane of a neuron, or of a lipid bilayer, actually induces a lipid phase transition? Thus transmembrane potential changes seems to be an area that has not so far received much attention by these workers; certainly they have not reported any results of tests that confirms the ability of small membrane potential depolarizations to induce lipid phase transitions in the plasma membranes of neurons or in lipid bilayer systems.

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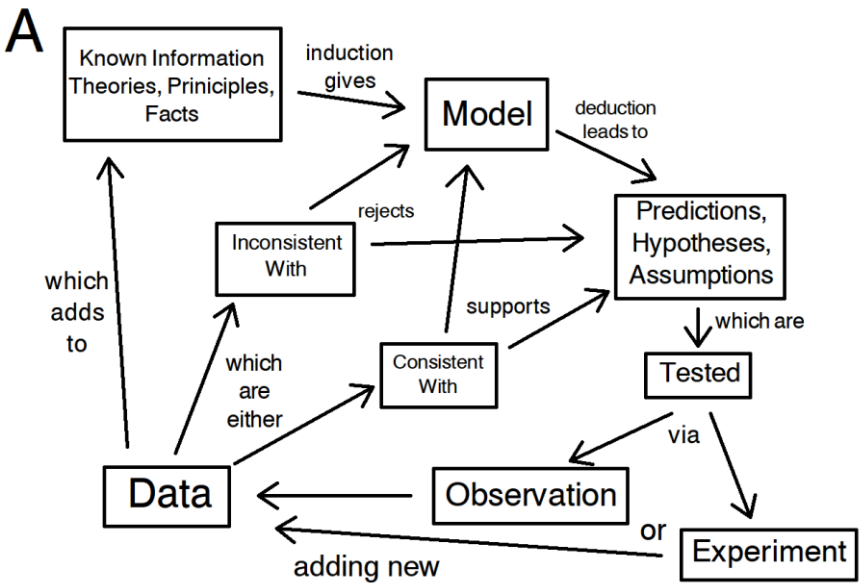

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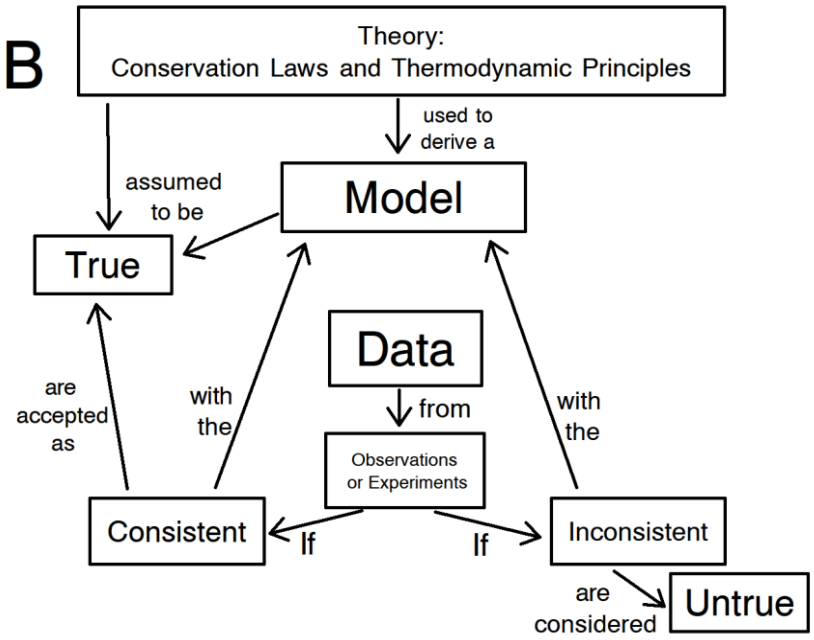

Figure 3. Concept maps comparing the standard Scientific Method with that of the new Thermodynamic/Theory-Based philosophical approach. A.) For the Scientific Method, showing how there is use of testing of a model's hypotheses by production of new data for use to evaluate the model. Modified from Glase (2002, Fig. 1.2). B.) And for the Thermodynamic/Theory-Based philosophical approach, showing how the model is used to

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evaluate data, with data that are consistent with the model being accepted, and data inconsistent with the model being rejected or ignored.

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4. A new philosophical approach.

What the advocates of the soliton/wave-AP model have done is not just propose a new model. Rather they are presenting a philosophical approach that differs from that of the scientific method. Next, after a brief review of the scientific method, a general outline of this new thermodynamic/theory-based philosophical approach will be given. Then, specific examples of its use in practice will be described in terms of how evidence is evaluated relative to the soliton/wave-AP model. The use of the Newtonian maxim under this new philosophical approach will be considered as well. And then a comparison of this new thermodynamic/theory-based philosophical approach to other approaches from before the rise of the scientific method will be made. It will be argued that this thermodynamic/theory-based philosophical approach is radically different from the scientific method, uses a very different standard of evidence, and that it is unlikely to encourage the carrying out of the sort of critical tests of its assumptions and predictions needed to convince those who work under the scientific method to accept the soliton/wave-AP model.

4a. The Scientific Method.

For the past several hundred years the scientific method has stood out as one of the most successful scientific philosophical approaches ever devised (Sanford 1899; Westaway 1919; Glase 2002; Popper 2005; Kosso 2011; Wagensberg 2014). While it shows flexibility when applied in specific contexts (Cleland 2001), it can be outlined simply as a pattern of practices and standards of evidence that are commonly used by scientists today (Fudge 2014). Put briefly (Fig. 3A); using existing knowledge, both factual and theoretical, new models are devised and used as broad research hypotheses. Each model makes specific predictions, or depends on specific assumptions, which are then subjected to testing either through gathering new observations or by obtaining new results from specific experiments. These new data are then used to evaluate the original model with several possible outcomes. The data may support the original model, and this then adds some additional confidence to that model. Or the data may be inconsistent with the original model, and so the model either needs to be modified or rejected. Or the data may be shown to be itself in error or the result of an invalid test. Thus under the scientific method the model is tested by the data. Also the scientific method encourages a consideration of alternative hypotheses (Platt 1964), the refutation of alternatives being one means by which confidence in the remaining hypothesis can be enhanced.

Thus, under the scientific method it is expected that all models, even those we call the laws of conservation, should be open to being questioned and tested against new data. Indeed, Crivellin et al. (2021) notes that the standard model of physics, one of the most

 doi:10.20944/preprints202208.0248.v1

successful of physical models, is open to such questioning and testing. This testing of such laws is not done because of major doubts about the laws of conservation, but rather the testing is often needed because the application of these laws is done by people who may be mistaken in the ways they apply them, or because in new situations new extensions, limitations, or features of the existing laws may thus be discovered, as might entirely new laws and principles. Therefore, questions about, and testing of, the modern electrophysiological-AP model, as derived from the past Hodgkin-Huxley-AP model, are welcome as are tests of all existing models.

So when the good folks who advocate for the soliton/wave-AP model propose that there is no change in permeability to specific ions during an action potential, that no ion movements occur across the membrane during an action potential, or that there is no net heat resulting from AP firing, the proper practice under the scientific method would be for them to repeat, or improve upon, past experiments and present new data that support their claims. However, the advocates for the soliton/wave-AP model have neither made such tests, nor presented such new data. They state their claims, but present no new evidence to back them up. Thus under the scientific method these, and many of their other claims about flaws in the modern electrophysiological-AP model, would be considered at this point to be mere hypotheses, (*i.e.*, theoretical conjectures) which have yet to be supported by needed data resulting from specific tests or observations. On the other hand, with regard to the soliton/wave-AP model that these good folks bring forward, there is already much data from the past that apparently refutes many of this model's hypotheses. Again, under the scientific method, it is appropriate for the advocates of this new model to carry out new critical experiments and bring forward new data. But, so far, the advocates to the soliton/wave-AP model have either not yet done the critical experiments, or have not made the needed key observations, and so have not managed, yet, to find the needed new evidence in support of their model so that under the scientific method their soliton/wave-AP model can be seen as more than a theoretical conjecture. Thus modern scientists expect the scientific method to be carried through to completion, so that the model can be judged by new data, and this the advocates of the soliton/wave-AP model have yet to carry through in force. This may then account, at least in part, for the lack of wide acceptance of the soliton/wave-AP model, and so the failure of this model to be accepted may not be just due to a misunderstanding about the nature of sound waves as suggested by Shrivastava (2021). Rather it seems to be a failure to use the scientific method to test the hypotheses put forward, and to present new evidence that confirms the many claims this model makes, which seems to limit its acceptance.

None of the above should be taken as dismissive of the findings made by the advocates of the soliton/wave model with regard to the phenomena they have documented concerning waves and lipid phase changes in simple lipid monolayers and bilayers. That work is often sound and largely of great interest. What is questionable

 doi:10.20944/preprints202208.0248.v1

is the assumption that, because they have found that lipids under one set of conditions can show these sorts of phenomena, then this must account for similar phenomena in all biological membranes as well. Thus the arguments presented earlier in this article were made to point out that relationships that might be confirmed in simple phospholipid bilayers might not extend to the much more complex setting of biological membranes. And the points raised in earlier work (Meissner 2018; Peyrard 2020) indicate that many of the assumptions these good folks are making in claiming that their soliton/wave model accounts for action potentials in excitable cells need testing. Such assumptions, under the scientific method, can and should be expected to under go intense testing because they are attempting to apply the relationships they discovered in one context to a drastically new setting without having yet done the needed tests to confirm that these relationships still hold up under these new conditions. Thus, the advocates of the soliton/wave-AP model may, under the scientific method, enhance the confidence in their arguments by presenting results of tests that support predictions of their model. For instance: Are lipid phase transitions actually happening during AP firing in excitable cells? If so, can shifts in surface potentials during such lipid phase transitions truly account for the macroscopic transmembrane potential changes we call an action potential? These, and many other issues have not had supportive findings reported, and under the scientific method such findings are absolutely needed in order to establish confidence in a proposed model.

4b. The Thermodynamic/Theory-based Philosophical Approach.

But the advocates of the soliton/wave-AP model have gone beyond just suggesting new hypotheses, they also are using in their studies a very different philosophical approach compared to the scientific method. This thermodynamic/theory-based philosophical approach has many distinct features, and so it merits our special attention.

The advocates of this thermodynamic/theory-based philosophical approach have recently described some of its philosophical aspects (Kang et al. 2020; Drukarch et al. 2021; Fabiunke et al. 2021; Fillafer et al. 2013, 2021; Mussel et al. 2021; Schneider 2020, 2021; Shrivastava 2021). They start with the premise that certain scientific laws and principles are to be taken as solid and to always be valid in all situations. From this starting point, models and formulae may be derived that follow directly from these laws and so, they argue, must themselves be considered valid under broad conditions. They thus hold the view that this thermodynamic-approach produces "correct predictions" (Andersen et al. 2009, pg. 107), and hold that to reject or question their model would be then to reject the principles and laws on which it is based. This would be in effect, they argue, to ignore the implications of the second law of thermodynamics (Heimburg 2019; Drukarch et al. 2021), or the consequences of the

law of conservation of momentum (Schneider 2020, 2021) (though for thoughts on how our views of the momentum of waves in a physical medium might need to be limited see McIntyre 1981). Typical is the following statement by Fichtl et al. (2018, pg. 4914) who state: "Importantly, this is not a hypothesis but is inevitable, following directly from the second law of thermodynamics." This approach has the interesting outcome that the resulting model is then presume to be "true" as they argue it is so closely associated with the underlying laws and principles that the authority of those laws extends to grant authority to the model (Fig. 3B), and so the model then must be "correct," as must then be its implications. In presenting their philosophy Drukarch et al. (2021) argues that there is a need for such a strong theoretical basis, and implies that empirical approaches are likely to produce misleading outcomes. How this philosophical approach is then applied relative to past or new data becomes rather interesting. In this regard Drukarch et al. (2021, pg. 6) indicates that primacy is given to theory when they state: "However, if a hypothesized constitutive element of the constructive theory... is found in contradiction with the theory of principle the hypothesized constitutive element will have to be dropped as it would amount to violation of the second law of thermodynamics." Thus under this thermodynamic/theory-based philosophical approach the model is assumed to be valid, and any data, either experimental or observational, has its validity largely judged based on its relationship to the model (Fig. 3B). Findings that are inconsistent with the presumably "correct" model are judged to be untrue or invalid in some manner, and only findings that are consistent in some way with the model are accepted. Also existing evidence often has to be reinterpreted so that it can be viewed in a manner that is consistent with the presumed "correct" model. To illustrate how this approach plays out in their arguments for their soliton/wave-AP model, some examples will next be offered.

4c. Applications of the thermodynamic/theory-based philosophical approach.

As noted previously, one of the major features of the electrophysiological-AP model is that during an AP the membrane has a transient and specific change in permeability for certain ions which are then able to move down their electrochemical potential gradient and so produce the pattern of changes in the membrane potential we call an action potential. But, under the soliton/wave-AP model it is assumed that no change in membrane permeability occurs. And so when Mussel et al. (2019a, pg. 4) states: "... by ignoring the effect of permeability on
the transmembrane potential measurement, the large contribution of surface potential is highlighted..." not only are they sweeping aside a large body of evidence that demonstrates that changes in membrane permeability occur during an AP, but they are also ignoring the consequences of the law of conservation of charge. This is done, apparently, because the soliton/wave-AP model does not require such changes in permeability, and therefore since their model is presumed to be "correct" these

 doi:10.20944/preprints202208.0248.v1

permeability data which contradict it must themselves be viewed as incorrect (Fig. 3B). Notice that the good people who advocate for the soliton/wave-AP model have not presented any new measurements which support their claim that during an AP the permeability of the membrane to ions does not change. Under the scientific method such new data, if shown to be repeatable, would likely be viewed with great interest. Instead, arguing from their thermodynamic/theory-based philosophical approach these good folks are using the model to decide which data are relevant. Thus, the model judges the data.

As another example, as noted previously, in examining phase transitions in DMPC bilayers it was found that with the addition of 50 mol% cholesterol no evidence could be detected of any lipid phase transition by differential scanning calorimetry (Peters et al. 2017). This is a finding highly relevant to the soliton/wave-AP model, which claims that a lipid phase transition is responsible for the features we call an action potential in the plasma membranes of neurons. But the plasma membrane of neurons has a high concentration of cholesterol, and so the finding by Peters et al. (2017) of no phase transition in bilayers that have a high content of cholesterol, a content similar to that expected in the plasma membrane of neurons, implies that something is wrong as the soliton/wave-AP model requires that such phase transitions happen. Therefore, since under the thermodynamic/theory-based philosophical approach the model is presumed to be "correct" the authors of Peters et al. (2017) suggest that the results they obtained were an artifact. They suggest that somehow differential scanning calorimetry in this case failed to detect a lipid phase transition they presume to be happening. This illustrates how the soliton/wave-AP model is used to judge the data, and, it seems, the data have to be consistent with the model in order for that data to be valid under this thermodynamic/theory-based philosophical approach. Contrast this with the results described previously from Mužić et al. (2019) where using a crude homogenate differential scanning calorimetry data were obtained which might indicate a possible lipid phase transition could happen. In this case the authors suggest that differential scanning calorimetry is a good method for detection of such phase transitions in plasma membranes known to have a high cholesterol content. In contrast, the Peters et al. (2017) article suggests that in the presence of high cholesterol content differential scanning calorimetry might not be able to detect a phase transition. So when the results are consistent with their model Mužić et al. (2019) suggest that the differential scanning calorimetry method used works well, but when not finding what the model expected Peters et al. (2017) suggests that the differential scanning calorimetry method does not work and is somehow missing a lipid phase transition that their model suggests must be present. Thus if the results are consistent with the presumed "correct" soliton/wave-AP model they are accepted, but if the results are in contradiction with this presumably "correct" model then they are rejected. Thus under the thermodynamic/theory-based philosophical approach, the model judges the data. The alternative, which the good people who advocate for the

 doi:10.20944/preprints202208.0248.v1

soliton/wave-AP model do not seem to consider, might be that the model they present, and the relationships upon which it is based, only operate under certain conditions, and so these relationships may not be truly as universal as they claim?

This pattern of using the model to judge the data is also seen in how the good people who advocate for the soliton/wave-AP model argue that no transmembrane movements of ions or changes in membrane permeability are needed during an AP (Heimburg et al. 2006). They argue instead that the voltage changes seen during the AP are due to changes in surface potentials that arise with the soliton/wave associated lipid phase transition. In support of this, the good Professor Heimburg (2018), inspired by a report by Tamagawa et al. (2018), suggests that changes in surface charge density, and so changes in surface potential, on the two sides of the plasma membrane during a presumed lipid phase transition might cause local ion adhesion, and alter the transmembrane potential. This is said to be similar to the suggestion made by Tamagawa et al. (2018) that in the two cells of a battery surface charge density changes in the battery half-cells may account for the potential differences between the cells, without the need for the flow of any ions between the two cells, and indeed Heimburg (2018, pg. 866) refers to the wire connecting the two cells of the battery as an "impermeable wall" as no ions can pass through it. Tamagawa et al. (2018) go on to show how based on surface ion absorption it is then possible to derive an equation very similar to the Goldman-Hodgkin-Katz equation, but which requires no movement of ions across the barrier at all. Based on this, Heimburg (2018, pg. 866) claims that: "Here, voltage changes are a consequence of variations in capacitance and polarization, and no selective permeabilities for ions are required." What is not being considered here, apparently, is that the wire is indeed selectively permeable in that it does allow some charged items to pass rather freely (*i.e.*, electrons) while still being an effective barrier to the passage of other charged items (*i.e.*, ions). Thus, the wire is not the "impermeable" barrier to all charge carrying items as Heimburg (2018) seems to suggest. In this battery system the difference in redox potential between the two half-reactions in the two battery cells creates an electron motive force which drives electron movement through the wire, and the charge carried by the movement of the electrons (which is a current we can measure) thus creates the electrical potential between the two battery half-cells, as is described in any good college chemistry textbook (Pauling 1957). This battery system is then clearly analogous to how certain charge-carrying ions move through ion-selective channels in response to specific electrochemical gradients across biological membranes, with both the battery and membrane systems each having a selectively permeable barrier (*i.e.*, the wire, versus the ion-channels), current (carried by either electrons, or ions), and a driving force (either a redox potential difference driving electron flow, or a specific ion's electrochemical potential difference). Thus, it is not surprising that analogous equations describing these two systems can be derived as both of these are systems with selectively permeable barriers. What is of interest here is the

 doi:10.20944/preprints202208.0248.v1

way that, by ignoring the flow of charged items between the cells of the battery, Heimburg (2018) is suggesting how this system should be viewed so that it seemingly supports the contention made by the advocates of the soliton/wave-AP model that there is no need for movement of any ions across the membrane of an excitable cell to create the pattern of transmembrane potential changes seen during the firing of an action potential. Thus, this is the sort of argument that is made in order to fit the data to what is presumed to be the "correct" model.

Similarly, reports that the action potential is dissipative, displays net heat, and so is dependent on the expenditure by the cell of metabolic energy to be sustained (Ritchie, 1973; Crotty et al. 2006; Magistretti et al. 2015; Yi et al. 2016), are also in contradiction to the presumed "correct" soliton/wave-AP model which is argued to have no net heat associated with it at all and to be adiabatic (Heimburg 2021), and so results such as those presented by Ritchie (1973) are rejected. Thus when starting with an assumed "correct" model these good people view data through a different standard of evidence than would those who follow the now commonly used scientific method-based philosophical approach. Indeed, Heimburg (2021, pg. 36) goes so far as to state: "Within experimental accuracy, no heat is dissipated during the action potential (Abbott et al., 1958). This indicates that no metabolism occurs during the nerve pulse." However, the article cited here, Abbott et al. (1958, pg. 157), states: "The positive heat averaged 8.8 x 10^{-6} cal/g, the negative heat 6.8 x 10^{-6} cal/g, and the net heat 2.0 x 10^{-6} cal/g." Thus, Heimburg (2021) is ignoring the conclusion reached by Abbott et al. (1958), that there is net heat associated with an action potential. This conclusion by Abbott et al. (1958) is clearly inconsistent with the soliton/wave-AP model's contention that action potentials are adiabatic, and so should produce no net heat. Thus, this finding of net heat by Abbott et al. (1958), being inconsistent with the soliton/wave-AP model, is ignored, and replaced with a conclusion that would be supportive of the soliton/wave-AP model. A similar reworking of reported information was done earlier when in the Heimburg et al. (2005, pg. 9794) article they claimed: "In particular, data indicate that heat release is exactly in phase with the action potential (12, 13), and that there is no net heat release after completion of the action potential." Here one cited item (number 12) is the Howarth et al. (1968, pg. 745) article which states: "At about 5°C the measured positive heat is 7.2 μcal/g. impulse.... The measured negative heat at about 5°C is 4.9 μcal/g. impulse." In this case, since the positive heat is greater than the negative heat, this implies a net heat emission results from an action potential, which directly contradicts what Heimburg et al. (2005) states. Yet Heimburg et al. (2005) cites Howarth et al. (1968) in support of their contention of action potentials producing no net heat. Thus the previously published data are made to fit to the presumed "correct" model.

 doi:10.20944/preprints202208.0248.v1

Another example of how the presumed "correct" model acts as a filter, may be see in how Heimburg (2021) deals with attempts to account for the pulses of first positive and then negative heat seen during action potentials. Heimburg (2021) notes that attempts have been made to account for this pattern of heat emission and absorption by a consideration of the charging and discharging of the system's capacitance due to the flow of ions associated with the action potential, which some call the "condenser" model. He notes that an analysis by Howarth et al. (1968) found that this capacitative effect would only account for about half of the magnitude of the heat emission and absorption observed, and so seems inadequate. However, that analysis by Howarth et al. (1968) treated the plasma membrane as having similar faces, with similar composition and similar surface charge density. Another study, which is noted by Heimburg (2021) to be an interesting thermodynamic analysis of this issue, was recently done by de Lichtervelde et al. (2020). de Lichtervelde and colleagues noted that the faces of a typical plasma membrane are not similar, rather the faces differ in composition and the inner face of the membrane typically has more negative surface charges per area than does the outer face. When de Lichtervelde et al. (2020) then redid the analysis for the condenser model in the context of such an asymmetric membrane they found that given the ion flows typically seen during an action potential such a membrane might well be expected to produce heat emission and absorption of the magnitudes reported to be observed. Thus, de Lichtervelde et al. (2020) found that this pattern of heat flow seen during an action potential might well be consistent with a condenser-type model, and so with the modern electrophysiological-AP model. What is rather odd, is that, having noted this work by de Lichtervelde et al. (2020), then Heimburg (2021, pg. 37) goes on to conclude: "... the condenser theory has been dismissed by most authors because the measured heat changes are significantly larger than what could be explained by charging of a capacitor using known capacitance values and using the voltage changes that have been measured." Thus, even when given a plausible means to account for such a pattern of heat flows by de Lichtervelde et al. (2020), one that is even based on theoretical thermodynamics, Heimburg still ignores the approach. This, apparently is done as the soliton/wave-AP model is taken to be the "true" model and so its accounting of this heat emission and absorption during an action potential by a presumed lipid phase transition must be seen as the "correct" prediction. Thus, under the thermodynamic/theory-based philosophical approach only the implications from the model that is presumed to be "correct" should be considered, and other alternatives are not to be taken seriously.

Let us consider some additional examples of how the items presumed by the soliton/wave-AP model, or supportive of it, seem to be accepted without much actual evidence in their support. Recall that in Mužić et al. (2019, pg. 2) they state: "Lipid melting peaks and protein unfolding profiles can easily be distinguished in pressure calorimetry due to their characteristic pressure dependencies, the pressure dependence of

 doi:10.20944/preprints202208.0248.v1

lipid transitions being much higher than that of proteins [21]." Here reference 21 is the work of Ebel et al. (2001). But on looking at Ebel et al. (2001) no data are presented in that article which relates to differences in how proteins versus lipids would have their calorimetric data altered by various applied pressures. Thus the claim Mužić et al. (2019) is making here seems to not be explicitly supported by the cited item? Similarity, in Fillafer et al. (2021) they note that their soliton/wave-AP model assumes that lipid phase transitions do occur commonly in eukaryotic plasma membranes, but with regard to evidence in support of this assumption they note (Fillafer et al. 2021, pg. 58): "However, the basis of evidence is too small, indirect and scattered for systematic conclusions." They then attempt to fill this void by citing several examples which they apparently feel do support this assumption, that lipid phase transitions are common and adaptive in eukaryotic membranes. But on close examination, several of the items they cite seem to actually argue against the very assumption Fillafer et al. (2021) claim they support. For instance, they cite Crowe et al. (1989) who reported the detection of lipid phase transitions upon the cooling of cattail pollen and of some animal sperm. But Crowe et al. (1989) note that these lipid phase transition events are associated with damage to the cells, which seems to argue against such phase transitions being adaptive as Fillafer et al. (2021) seem to wish to claim. Similarly, Fillafer et al. (2021) cite a review article by Melchior et al. (1976) in support of the notion that lipid phase changes occur commonly in eukaryotic biological membranes. But Melchior et al. (1976, pg. 226) state: "But the bulk thermotropic transition and lateral phase separation as seen by experimental methods now employed appear to be unnecessary for the life of the cell at growth temperature. On the contrary, it is evidently an effect to be avoided. It is accompanied by a variety of usually undesirable physiological events, and it is clear that living systems take pains to lower their transition ranges to acceptable temperatures." So again, an item that Fillafer et al. (2021) cites in support of lipid phase transitions in excitable eukaryotic cells turns out to actually argue that such phase transitions are normally rare as they are often maladaptive. Finally, Fillafer et al. (2021) also cites Inoue et al. (1973) in support of the notion of lipid phase transitions in excitable cells. But what Inoue et al. (1973) actually refer to are transitions in macromolecules, which might be taken to mean proteins in the membranes undergoing conformational changes as lipids are not macromolecules? This view is supported where Inoue et al. (1973, pg. 476) state concerning changes in the plasma membrane during action potential firing: "The abrupt and discrete conformational changes are considered to represent phase transitions in the membrane macromolecules." Changes in protein macromolecular conformations during an action potential are, of course, fully consistent with the modern electrophysiological-AP model, as reflected in conformational changes that would occur in voltage-sensitive protein-based ion channels in the plasma membrane. Thus what Inoue et al. (1973) present seems not truly in support of a lipid phase transitions, but seems rather to be in support of protein conformational changes. Thus Fillafer et al. (2021) seem correct to suggest that the

 doi:10.20944/preprints202208.0248.v1

evidence for the soliton/wave-AP model's presumed lipid phase transition is "small," but what is of interest here is how Fillafer et al. (2021) seemingly are viewing the information in many of the items they cite through the filter of their presumably "correct" model, and so end up claiming support for lipid phase changes that seems to not actually be there. This is perhaps an illustration of what may happen under the thermodynamic/theory-based philosophical approach when the data have to be viewed in a way so that they will conform to a model that is presumed to be "correct."

Another example of this use of the soliton/wave-AP model to judge the data can be seen with regard to the findings of Lee et al. (2017). Lee et al. (2017) made use of Raman scattering spectroscopy done on a neuron which was firing APs, and found evidence of signal changes associated with protein shifts during the APs. It may be noted that others have used Raman scattering spectroscopy to follow changes in protein conformation in other contexts (Hildebrandt et al. 1990). Lee et al. (2017) attribute some of the signal they detect to changes in conformation of the sodium voltage-gated ion channel during action potentials, and they note that they did not find evidence of changes associated with signals from the lipids during action potentials. This lack of any indication by this method of a change in lipid arrangements during AP firing confirms what was reported previously by Pézolet et al. (1985) and by Savoie et al. (1986). These results from Lee et al. (2017) may be taken as supportive of the modern electrophysiological-AP model, which very much depends on conformational changes in voltage-gated ion channel proteins in the plasma membrane during action potentials. It may also be noted that Sonnleitner et al. (2002) had previously reported the ability to detect the conformational changes of fluorescence probe-labeled ion channels in response to changes in membrane potential, which may be consistent with the findings of Lee et al. (2017). However, a preprint posted by Shrivastava et al. (2020) characterizes the data from Lee et al. (2017) as supporting membrane melting during action potentials, which they suggest should be seen as a "thermotropic transition in the membrane" (Shrivastava et al. 2020, pg. 5) during the action potential. Thus, Shrivastava et al. (2020) argue that these reported data should be seen as consistent with the soliton/wave-AP model, which requires that some sort of lipid phase transition occurs. What Shrivastava et al. (2020) is suggesting is that during an AP a change in the lipid arrangements (which Lee et al. (2017) did not report detecting) is actually happening, while the indications of changes in specific proteins (which Lee et al. (2017) did detect) are then ignored by Shrivastava et al. (2020). Here, once again, data which would seem to contradict the soliton/wave-AP model, by finding no clear evidence of a lipid phase transition, are reinterpretated as actually supporting the occurrence of the soliton/wave-AP model's presumed phase transition. Mussel et al. (2021) have since refered to Shrivastava et al. (2020)'s argument as being: "The first work claiming for detection of a lipid phase transition occuring during an action potential was only recently published, and used Raman spectroscopy (Shrivastava et al., 2020)"

 doi:10.20944/preprints202208.0248.v1

(Mussel et al. 2021, pg. 108). Thus, even though the Lee et al. (2017) did not report any evidence that indicates such a lipid phase transition was happening during action potential firing, the advocates for the soliton/wave-AP model still refer to this work in support of something it did not actually find. Thus, under the thermodynamic/theory-based philosophical approach the data must be forced to fit the model.

Nor is this the only matter in which Mussel et al. (2021) seem to reinterpret the findings reported by others. Mussel et al. (2021) notes a theoretical study by Yagisawa et al. (1993) in which it is suggested that a self-sustaining oscillation of phase transitions might be achieved in a phospholipid bilayer system. What Yagisawa et al. (1993) presented was a theoretical hypothesis, as a model, which if confirmed in an actual lipid bilayer system might indeed lend some interesting aspects to the soliton/wave model. This hypothesis was tested by Srivastava et al. (1998) in actual lipid bilayers and about their work Mussel et al. (2021, pg. 108) states... "Nevertheless, self-sustained oscillations of electrical potential difference across a synthetic lipid bilayer were demonstrated (Srivastava et al., 1998)." However, what Srivastava et al. (1998, pg. 75) actually reported was: "The data do not corroborate the postulate of gel-liquid crystal phase transition, induced by the repetitive adsorption and desorption of protons by the membrane surface... The model [presented by Yagisawa et al. (1993)] though theoretically self consistent does not seem to correspond to the actual reality at least in the liquid membrane bilayer system presently studied." This then is what Mussel et al. (2021) cite in support of the notion that phase transitions can be self-sustaining. However, they make a claim here that directly contradicts the actual findings of Srivastava et al. (1998), and in doing so they show, once again, how under this thermodynamic/theory-based philosophical approach the data must be fitted to the presumed "correct" model, even, apparently, if that means directly contradicting what previous researchers conclude about their own findings. This perhaps indicates the level of confidence some of those good folks who advocate for it place in their soliton/wave-AP model?

Lastly, one particularly interesting example of how this thermodynamic/theory-based philosophical approach is applied with regard to the soliton/wave-AP model can be seen in an instance where an initial claim actually ended up being modified. What actually caused this modification of the soliton/wave-AP model in this case is of interest. The specific instance, described more fully previously (Meissner 2018), is with regard to the claim made by the proponents of the soliton/wave-AP model that two action potential moving in opposite directions towards each other and which collide would, as waves, pass through each other and not annihilate (Appali et al. 2012; Gonzalez-Perez et al. 2014, 2016; Lautrup et al. 2011; Vargas et al. 2011). The fact that in Gonzalez-Perez et al. (2014) they actually reported observation of annihilation of colliding action potentials in some cases apparently was not sufficient for them to consider their hypothesis to be in

 doi:10.20944/preprints202208.0248.v1

doubt. Thus, a presumed theory-based model in which they instill great confidence was not to be questioned by the mere finding of apparently contradictory observations. Neither of great influence were the comments by Berg et al. (2017) who noted that action potential annihilation has been commonly observed by neurobiologists, and indeed is commonly used in mapping neuronal connections. In response to all of this apparently disconfirming evidence, some of the advocates of the soliton/wave-AP model replied that such reports of action potential annhibilation needed to be reevaluated (Wang et al. 2017). Thus the confidence these advocates had in their soliton/wave-AP model was such that they would rather put faith in their model than consider disconfirming evidence. This seems to be a common feature of the thermodynamic/theory-based philosophical approach, in that the model is used to judge which specific evidence should be accepted or rejected. What makes this example especially interesting is that more recently some advocates of the soliton/wave-AP model have shifted their views on this matter, and now allow that colliding action potentials may indeed be able to under go annihilation (Drukarch et al. 2021; Mussel et al. 2021). This altering in their stance was not brought about merely by any new evidence from experiment or observation. Rather this alteration seems to be mainly the result of the devising of a means to account for action potential annihilation via a theoretical approach (Shrivastava et al. 2018b). Thus, under this thermodynamic/theory-based philosophical approach the models presented can be altered if there is a basis in theory to justify the alteration. However, apparently mere observational or experimental results are not granted sufficient authority under this philosophical approach to lead to any significant judgement being made of a model. This confirms that this thermodynamic/theory-based philosophical approach is using a very different standard of evidence compared to the scientific method where models would be judged by observational and experimental evidence (Fig. 3).

4d. On the uses made of the Newtonian maxim.

Another interesting aspect of this thermodynamic/theory-based approach is the notion that there can only be one cause for a given phenomenon (Drukarch et al. 2021; Schneider 2021). This is seen in their use of a Newtonian maxim, which is presented in Fillafer et al. (2016, pg 363) as follows: "We are to admit no more causes of natural things than such as are both true and sufficient to explain their appearances. Therefore, to the same natural effects we must, so far as possible, assign the same causes." Thus under this Newtonian maxim once an explanation is achieved by these advocates of the soliton/wave-AP model through their theory-based approach, then no other causal explanation need be considered leaving their theory-based explanation as the only acceptable view. All other alternatives are therefore to be rejected.

 doi:10.20944/preprints202208.0248.v1

As an example of the application of this Newtonian maxim we may look to the argument given by Schneider (2021) with regard to the existence of protein-based ion channels. Such channels are, of course, central to the operation of the electrophysiological-AP model. However, the soliton/wave-AP model assumes that there is no movement of ions across the plasma membrane through ion channels during an action potential (Appali et al. 2012; Gonzalez-Perez et al. 2014; Heimburg 2010; Vargas et al. 2011). This argument is carried further when Schneider (2021) applies the Newtonian maxim to this issue and claims that the mere existence of lipid pores that form during lipid phase transitions would account for any movements of ions that might occur and so, given a cause for the phenomenon of ion flow across the membrane, no other means or causes for ion flow across membranes should be sought or considered; an argument that is also found in other articles by the advocates of the soliton/wave-AP model (Kang et al. 2020; Zecchi et al. 2021). Though it should be recalled that the formation of such lipid pores has been reported to be damaging to cells (Pakhomova et al. 2014; Meissner 2018). So having pointed out that transmembrane ion flow can be accounted for by lipid pores that appear in association with lipid phase transitions without any proteins being present, Schneider (2021, pg. 5) then states the following conclusion: "Hence, the hypothesis [of protein-based ion channels] is falsified..." Thus, given their confidence in the soliton/wave-AP model, based on its presumed foundation in basic conservation laws and thermodynamic principles, this rejection of the existence of protein-based ion channels is further argued to be indicated by this application of the Newtonian maxima apparently because there is another means for ions to pass through bilayers and membranes.

Of course, there is much evidence in favor of the existence of protein-based ion channels. A review by de Lera Ruiz et al. (2015) of what is known about just one category of voltage-sensitive sodium ion channels notes that over a two year period well over a thousand articles were published on just this one category of ion channels! Information about these and many other types of ion channels, as well as methods used in their study, is noted by McClintock et al. (2018) to be collected in the CRC text "Handbook of Ion Channels." One study, Holzenburg et al. (1993), which examined the structure of an ion channel protein, is perhaps notable as Prof. Heimburg was a coauthor. The good Prof. Schneider (2021), in claiming that the existence of such ion channels has been "falsified," does not state explicitly what he considers to be wrong with the body of the current evidence for the existence of protein-based ion channels, nor does he suggest any type of evidence he would accept that might cause him to consider that such ion channels actually exist. Rather Schneider (2021) is claiming, through a strict application of the Newtonian maxim, that evidence for one way that ions can move across a membrane or bilayer should preclude consideration of any evidence of other ways to achieve such movement. Thus only one cause is to be accepted, and all other alternatives are to be rejected. In this case the one cause that is accepted by Schneider (2021) happens to be one consistent with the

 doi:10.20944/preprints202208.0248.v1

soliton/wave-AP model, as he claims it follows, in some manner, from the law of conservation of momentum (Schneider 2020).

Of course, logically, someone who has high confidence in the electrophysiological-AP model, perhaps because of its being based on relatively simple relations (Powell et al. 2021) which are connected to the laws of conservation of energy and of charge, might be tempted to apply the Newtonian maxim as a means to then argue against the soliton/wave-AP model? But under the scientific method such an argument would not be accepted as definitive, as under the scientific method multiple causes can exist for many phenomena. Rather, under the scientific method it might be argued that there is good evidence for soliton/waves in lipid monolayer and bilayer systems, but, so far, little evidence for them in the biomembranes of living cells. At the same time, under the scientific method, the modern electrophysiological-AP model can be accepted as a plausible basis for action potentials seen in excitable cells, while at the same time noting that such a model could not operate in pure lipid bilayers due to the absence of the needed protein-based ion channels. Thus what the scientific method allows is for similar patterns of events and phenomena to have different causes, especially in different contexts. And so under the scientific method both the soliton/wave model and the electrophysiological-AP model can be seen as essentially valid, but operating in different contexts such that there is no need to argue for one versus the other in some sort of competition. Thus, what is critical is that the thermodynamic/theory-based philosophical approach through its use of this Newtonian maxim does not promote an acceptance of there being multiple possible causes in different contexts, rather it tends to force a seemingly false choice to have to be made so that only one option is accepted. This is in stark contrast to how a careful consideration of alternatives is an essential aspect of good science as done under the scientific method.

Indeed, if we were to apply this Newtonian maxim strictly it would lead us to some rather unfortunate outcomes. For instance, if we first discover that proteins can engage in enzymatic catalysis, with that cause of catalysis in hand we would, under the Newtonian maxim, have to reject RNA-based enzymes (*i.e.*, ribozymes) as an additional cause of catalysis. Or, given the discovery that exposure of cells to ultraviolet radiation can lead to mutations in the DNA, under the Newtonian maxim knowing this cause of mutations should then preclude us from exploring chemical mutagens as an additional cause of mutations. Once ATP synthesis by substrate-level phosphorylation in glycolysis is known, then, under this Newtonian maxim, having this one cause established should preclude consideration of ATP production by the mechanism of chemiosmosis. Knowing that wildfires can be caused by lightning strikes should, then, preclude any consideration of wildfires being caused through human activities. Also, given the discovery that certain combinations of disease symptoms are caused through infection by a certain virus, such as viral influenza, then, under the Newtonian maxim, we should not look for other causes of

 doi:10.20944/preprints202208.0248.v1

these symptoms, and so the alternative that similar symptoms might result from infection with certain bacteria would not be open for consideration. Many other examples exist, the point being that many phenomenon are well known to have multiple causes, and so a justification for the use of this Newtonian maxim to limit consideration to just one cause seems lacking. Given that multiple causes are commonly encountered, how the advocates for the soliton/wave-AP model can argue that theirs is the only model that should be considered does not seem to be adequately justified by this flawed Newtonian maxim. In addition, their argument that the soliton/wave-AP model should be given primacy due to its connections to certain laws of conservation also seems uncompelling when it is recalled that the modern electrophysiological-AP model also has connections to several conservation laws.

This weakness in the Newtonian maxim has been noted by others. Westaway (1919, pg. 244) states the following on this matter: "But whatever Newton may have intended his *vera causa* precisely to signify, Mill is of opinion that Whewell has conclusively shown Newton's maxim to be wanting in both precision and self-consistency... At all events, it can hardly be considered necessary that the cause assigned should invariably be a cause already known; otherwise, we should sacrifice our best opportunities of becoming acquainted with new causes. It would be unreasonable to affirm that we already know all existing causes." The implication is that by its use of the Newtonian maxim the thermodynamic/theory-based philosophical approach is unnecessarily limiting in its consideration of alternative explanations. This is in stark contrast to the scientific method in which consideration of plausible alternative explanations is typically expected.

What is especially interesting about the application of this Newtonian maxim by the advocates for the soliton/wave-AP model is that often when they make a claim that there can be only a single cause, the cause they put forward as acceptable is the one offered by their model. And, it does not seem to matter just how much evidence exists for the rejected alternatives. Which suggests that this maxim is applied as a means to attempt to justify considering only one cause and to avoid consideration of any alternatives. Thus when Schneider (2021) claims under this single-cause Newtonian maxim that protein-based ion channels just should not be considered, notice how this leaves as the only alternative one that is more consistent with the soliton/wave-AP model. The same pattern is seen in the way that the advocates for the soliton/wave-AP model reject the role of ion flows during action potentials, or the production of net heat from AP firing, or many other alternative explanations that are consistent with the modern electrophysiological-AP model (Meissner 2018). These alternatives are rejected it seems because of a dogmatic adherence to a one-

 doi:10.20944/preprints202208.0248.v1

cause[2] mentality, which then allows these good people to avoid having to engage with the actual evidence for those alternatives. Thus the application of this Newtonian maxim seems to be done in a biased manner, so that their own soliton/wave-AP model is favored.

The application of this Newtonian maxim by the advocates for the soliton/wave-AP model also seems to be done somewhat inconsistently. This is seen in the arguments they make where, in suggesting how there might be induction of a lipid phase transition, upon which their model depends, they are willing to accept multiple possible causes. They argue that either changes in temperature, pressure, pH, or other items might all act, either individually or in some as of yet ill-defined combination, to cause the induction of a lipid phase transition in the plasma membrane of excitable cells (Kang et al. 2020; Drukarch et al. 2021; Fillafer et al. 2016, 2021; Schneider 2021). This acceptance of multiple causes is an apparent violation of their favored Newtonian maxim, but in this case they do not argue that the phenomena of lipid phase transitions should be limited to just one cause. Thus, their use of the Newtonian maxim is done to argue against alternatives to their soliton/wave-AP model, but when their own soliton/wave-AP model requires a consideration of multiple possible causes then they are quite willing, it seems, to ignore this Newtonian maxim. Therefore, this Newtonian maxim is being used as a rhetorical device (*i.e.*, a debating tactic if you will) by the advocates of the soliton/wave-AP model to attempt to sweep aside the alternatives to their favored model and to ignore the evidence for those alternatives.

4e. A neo-Aristotelian approach.

Here it is being argued that the advocates of the soliton/wave-AP model have devised a philosophical approach that is very different from that seen under the scientific method. Their approach assumes some sort of ideal, or irrefutable, background knowledge from which "correct" models can be generated. This then presumably "correct" model is used to judge which data fits with it and are to be accepted, and which data should be rejected or ignored (Fig. 3B). Those who have read something of the history of science will likely recognize elements of this thermodynamic/theory-based philosophical approach as being very similar to that used in the past. The Platonian approach assumed an ideal state, and the Aristotelian approach advocated for the fitting of data to the model (Lewes 1864; Westaway 1919). For instance, Lewes (1864, pg. 79) states: "The metaphysician and metaphysicist pretend to co-ordinate facts with all the rigor of a physicist; but they admit

[2] We might say: One cause to rule them all, one cause to find them, one cause to bring them all, and in the darkness bind them. In the land of lipids where the shadows lie. (With apologies to J.R.R. Tolkien (1973) for the abusive rephrasing of his fine work.)

 doi:10.20944/preprints202208.0248.v1

facts which have not withstood the preliminary test, and facts which are not amenable to that test. This disregard and misapprehension of the test are due to overweening confidence in the validity of reason. Ideas are accepted, unchallenged, as the correct representatives of the external order." The effects of the broad use of this sort of philosophical approach then need not be imagined, its effects are already displayed for us in scientific history. Indeed von Sachs (1906, pg. 171) describes these flawed early philosophical approaches in the following way: "Idealistic views of nature of all times, whether they present themselves as Platonism, Aristotelian logic, Scholasticism or modern Idealism, have all of them this in common, that they regard the highest knowledge attainable by man as something already won and established; the highest axioms, the most comprehensive truths are supposed to be already known, and the task of inductive enquiry is essentially that of verifying them; the results of observation serve to elucidate already received views, to illustrate already known truths; inductive enquiry has only to establish individual facts." Thus this philosophy is not focused on discovery of new principles, rather existing laws and principles are granted such authority that they are then used to organize new data. This flawed feature is also seen in the current thermodynamic/theory-based philosophical approach. This early philosophical approach led to stagnation, as can be seen across history in how this Aristotelian approach held investigations in such a trap of trying to confirm what was presumably already known, and so ignoring new features being offered up by reality, that for over a thousand years European science suffered a dark age. It was only with the coming of the scientific renaissance and the devising of the scientific method that European science began to reject metaphysical idealism and demanded that models and hypotheses be tested and judged by the collection of new data. Without confirmation of deductions, without testing of hypotheses and assumptions, no matter how firmly a model is thought to be based in theory, there is a risk of going astray. Westaway (1919, pg. 76) notes this in his description of the flaws of the Aristotelian approach: "Deductions drawn from unverified hypotheses are necessarily always open to doubt. The great danger of accepting such deductions was entirely overlooked by Aristotle, whose blunders, in consequence, are often grotesque."

Thus, it seems that the thermodynamic/theory-based philosophical approach is in essence a neo-Aristotelian approach, and it shares the flaw of a lack of rigorous testing of its assumptions and hypotheses as well as the presumption that the model should be given authority over data. To those who use the scientific method the certainty that the advocates of the soliton/wave-AP model place in their model may well appear to be over confidence. This too, has been noted to be a feature of earlier Aristotelian philosophy (Westaway 1919, pg. 28): "The fallacy is not usually in the actual chain of reasoning; philosophers do not often make elementary blunders of that kind. It is traceable rather to an untenable major premiss, adopted, perhaps, because of the royal confidence felt in some unexamined intuition, or because of some unsuspected prejudice..." As noted previously

 doi:10.20944/preprints202208.0248.v1

(Meissner 2018) one key assumption, and there are many others, that the advocates of the soliton/wave-AP model have made is that the phenomena associated with the lipid phase transitions they have documented in simple artificial lipid bilayer and monolayer systems must then mean that lipid phase transitions account for similar phenomena in the biological membranes of excitable cells. This they claim to be a valid connection, not because of actual evidence of such lipid phase changes happening in biological membranes, but because it is implied, they claim, by thermodynamics and other laws and principles. Thus this is a neo-Aristotelian manner of thinking, and shares many of the flaws and limitations seen earlier in history in similar approaches.

It is because of the philosophical approach we call the scientific method that we have managed to discern at all such things as the laws of conservation, the principles of thermodynamics, and a great may other patterns found in the universe around us (Westaway 1919; Ayala 2009). How ironic then, that after making this philosophical advance, and attaining this level of understanding of how to proceed productively in science, some would abandon the scientific method, and claim that we now know enough about the universe to allow us to revert to a type of metaphysical neo-Aristotelian approach, which seems to be what this thermodynamic/theory-based philosophical approach represents. But in using their thermodynamic/theory-based philosophical approach the good folks who advocate for the soliton/wave model risk stepping away from doing science, and towards doing doctrine. We might take to heart the words of Platt (1964, pg. 350) who when noting some areas of science seem to be advancing more than others stated: "Unfortunately, I think, there are other areas of science today that are sick by comparison, because they have forgotten the necessity for alternative hypotheses and disproof... This is not science, but faith; not theory, but theology."

5. In conclusion.

As noted previously, the soliton/wave-AP model has much of its support coming from studies of lipid bilayer and monolayer systems, and much of that work is very interesting indeed. It is rather fascinating to consider just what phenomena can be displayed by such relatively simple systems. And this naturally leads to questions of whether or not changes in lipid phases might have a functional adaptive role to play in biological membranes. Such questions are worthy of study. However, when the findings and relationships devised for such simple homogeneous lipid bilayer and monolayer systems are said to be universal, and the claim is made that these same relationships will operate in all biological membranes no matter their composition, and such claims are suggested to be beyond questioning, it then becomes the case that major reservations and questions arise (Peyrard 2020). Thus, the underlying questions being asked by the advocates of the soliton/wave-AP model are

 doi:10.20944/preprints202208.0248.v1

interesting and important, but the manner in which they are approaching these matters seems to have flaws.

This article has presented the argument that composition matters, and that specific interactions between specific membrane components influence what phenomena can occur, specifically in terms of whether lipid phase transitions and so soliton/waves should be expected to occur in eukaryotic membranes. The suggestion has been made that further study is needed of how soliton/wave phenomena may be limited to certain compositions, and so might be precluded from happening, or may occur in ways that are rather modified in yet other compositions compared to the pure DPPC bilayers upon which the soliton/wave model was largely originally based. It should also be noted that if across the normal physiological range of conditions no lipid phase transition occurs, as in a differential scanning calorimetry scan indicating a zero change in specific capacity relative to the baseline, that does not invalidate the equations devised by the good folks who have presented the soliton/wave-AP model. It merely may indicate that under certain conditions, when the ΔC_p is zero, that such soliton/waves may be unlikely to occur. It has been noted that this potential limitation of the soliton/wave system may be explored by the use of combinations of lipids in bilayer systems to examine the influence of composition. Testing has also been suggested to be needed in the matter of whether the changes in surface potential the soliton/wave-AP model suggests occur do actually have macroscopic influences. Thus, this article has questioned the claim of universality put forward by the good folks who advocate for the soliton/wave-AP model.

This article has also attempted to argue that for phenomena to be accepted as being used by living organisms the conditions under which the phenomena occur should fall within the normal physiological range. Phenomena that require conditions that are not within the normal physiological range of temperature, pH, or pressure may be argued then to be phenomena that we can induce artificially, but may not be phenomena that occur normally *in vivo* as the conditions needed to induce them are avoided by life as being damaging and so may seldom occur.

Perhaps the most important issue raised in this article, however, has to do with the philosophical approach used by the good advocates of the soliton/wave-AP model; the thermodynamic/theory-based philosophical approach. It has been argued that this approach shares many of the flaws that are found in metaphysical approaches used before the devising of the scientific method, and so is argued to be a neo-Aristotelian approach. And it has been argued that this thermodynamic/theory-based philosophical approach differs significantly in terms of operation and standards of evidence compared to the current scientific method.

 doi:10.20944/preprints202208.0248.v1

Under the scientific method the advocates of the soliton/wave-AP model are welcome to directly and fully examine the evidence for the existence and operation of protein-based ion channels, for the changes in permeability to certain ions that occurs during an action potential, and for the way that the net heat associated with an action potential is related to its dependence on metabolic processes and so is dissipative, etc. Thus, replication of the critical experiments that led to these and other findings, with perhaps improvements in methods, and the reporting of any findings that differ from those previously reported would be welcome. They also are welcome to carry out the proposed tests presented in this article, and previously in another (Meissner 2018), and present the results from them to show, if possible, that the many claims they make with regard to their soliton/wave-AP model are upheld. But the good people who operate under the thermodynamic/theory-based philosophical approach have largely not taken this approach, instead they offer claims that their model is "correct" in all essential ways, that its features are inevitable, and so is essentially irrefutable, they fit the data to their model, and they use rhetorical devices to avoid considering evidence that challenges their model. This illustrates how this thermodynamic/theory-based philosophical approach is quite different from the scientific method in terms of its standards of evidence.

Whether or not the advocates of the soliton/wave-AP model choose to continue using their current thermodynamic/theory-based philosophical approach, or whether they choose to use the modern scientific method, is for them to decide. But the choice they make will clearly matter in terms of the standards of evidence they use, and so may be expected to influence their attempts to argue effectively to the bulk of fellow scientists in favor of their soliton/wave-AP model. Clearly, most scientists expect the use of the scientific method and base their acceptance or rejection of hypotheses and models on the scientific method's philosophical approach and on its standards of evidence. Such a use of the scientific method should also involve a careful and rigorous examination of alternatives, and not merely dismiss them due to an assumption that a favored model is "correct." By using their different philosophical approach the advocates for the soliton/wave-AP model are making it far more difficult for themselves to produce a case capable of convincing those who use the scientific method of the validity of their model, as their thermodynamic/theory-based philosophical approach uses fundamentally different standards of evidence. This may also have the unfortunate outcome of causing there to be less investigation done into many of the interesting fundamental questions that these good people are raising. Therefore, these good people are urged to consider making full and robust use of the scientific method as the best means to advance our knowledge about many of the interesting possibilities they have raised.

In closing, as a reminder of the need for all scientists to consider alternatives (alternatives to the soliton/wave-AP model, as well as alternatives to the

 doi:10.20944/preprints202208.0248.v1

electophysiological-AP model) without bias or prejudice, a quote from Locke (as given by Westaway 1919, pg. 130) would seem appropriate:

"To those who would shake off the great and dangerous monster, prejudice, who dresses up falsehood in the likeness of truth, I shall offer this one mark whereby prejudice may be known. He that is strongly of any opinion must suppose that his persuasion is built upon good grounds, and that his assent is no greater than what the evidence of the truth he holds forces him to, and that they are arguments and not inclination or fancy that make him so confident and positive in his tenets. Now if, after all his profession, he cannot bear any opposition to his opinion, if he cannot so much as give a patient hearing, much less examine and weigh the arguments on the other side, does he not plainly confess it is prejudice governs him?"

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References:

Abbott B.C., A.V. Hill, J.V. Howarth- 1958-The positive and negative heat production associated with a nerve impulse- Proceedings of the Royal Society of London B, 148: (#931, 2/18) 149-187, https://www.jstor.org/stable/83038.

Ädelroth P., P. Brzezinski- 2004-Surface-mediated proton-transfer reactions in membrane-bound proteins- Biochimica et Biophysica Acta 1655: 102-115, doi :10.1016/j.bbabio.2003.10.018.

Aghaaminiha M., S.A. Ghanadian, E. Ahmadi, A.M. Farnoud- 2020-A machine learning approach to estimation of phase diagrams for three-component lipid mixtures- Biochimica et Biophysica Acta - Biomembranes 1862: 183350, https://doi.org/10.1016/j.bbamem.2020.183350.

Akemann W., A. Lundby, H. Mutoh, T. Knöpfel- 2009-Effect of voltage sensitive fluorescent proteins on neuronal excitability- Biophysical Journal 96: (May) 3959-3976, doi: 10.1016/j.bpj.2009.02.046.

Akiyama T., N. Kunishima, S. Nemoto, K. Kazama, M. Hirose, Y. Sudo, Y. Matsuura, H. Naitow, T. Murata- 2020-Further thermo-stabilization of thermophilic rhodopsin from *Thermus thermophilus* JL-18 through engineering in extramembrane regions- Proteins 89: 301-310, doi: 10.1002/prot.26015.

Alm I., S. García-Linares, J.G. Gavilanes, Á. Martínez-del-Porzo, J.P. Slotte- 2015-Cholesterol stimulates and ceramide inhibits sticholysin II-induced pore formation in complex bilayer membranes- Biochimica et Biophysica Acta 1848: 925-931, http://dx.doi.org/10.1016/j.bbamem.2014.12.017.

Almeida P.F- 2011-A simple thermodynamic model of the liquid-ordered state and the interactions between phospholipids and cholesterol- Biophysical Journal 100: (Jan.) 420-429, doi: 10.1016/j.bpj.2010.12.3694.

Almeida P.F.F., W.L.C. Vaz, T.E. Thompson- 1992-Lateral diffusion in the liquid phases of dimyristoylphosphatidylcholine/cholesterol lipid bilayers: A free volume analysis- Biochemistry 31: 6739-6747.

Al-Rekabi Z., S. Contera- 2018-Multifrequency AFM reveals lipid membrane mechanical properties and the effect of cholesterol in modulating viscoelasticity- Proceedings of the National Academy of Sciences U.S.A. 115: (#11, 3/13) 2658-2663, www.pnas.org/cgi/doi/10.1073/pnas.1719065115.

Amsalem M., C. Poilbout, G. Ferracci, P. Delmas, F. Padilla- 2018-Membrane cholesterol depletion as a trigger of Nav1.9 channel-mediated inflammatory pain- The EMBO Journal 37: e97349, 19 pgs., doi: 10.15252/embj.201797349.

Andersen S.S.L., A.D. Jackson, T. Heimburg- 2009-Towards a thermodynamic theory of nerve
pulse propagation- Progress in Neurobiology 88: 104-113, doi: 10.1016/j.pneurobio.2009.03.002.

Antonov V.F., A.A. Anosov, V.P. Norik, E.A. Korepanova, E.Y. Smirnova- 2003-Electrical capacitance of lipid bilayer membranes of hydrogenated egg lecithin at the temperature phase transition- European Biophysical Journal 32: 55-59, doi: 10.1007/s00249-002-0266-7.

Appali R., U. van Rienen, T. Heimburg- 2012-A comparison of the Hodgkin-Huxley model and the Soliton theory for the action potential in nerves- Chapter 9 in Advances in Planar Lipid Bilayers and Liposomes, 16: 275-299, http://dx.doi.org/10.1016/B978-0-12-396534-9.00009-X.

Arrais D., J. Martis- 2007-Bilayer polarity and its thermal dependency in the l_o and l_d phases of binary phosphatidylcholine/cholesterol mixtures- Biochimica et Biophysica Acta 1768: 2914-2922, doi: 10.1016/j.bbamem.2007.08.012.

Attwell D., SB. Laughlin- 2001-An energy budget for signaling in the grey matter of the brain- Journal of Cerebral Blood Flow and Metabolism 21: 1133-1145.

Ayala F.J- 2009-Darwin and the scientific method- Proceedings of the National Academy of Sciences U.S.A. 106: (S1, 6/16) 10033-10039, www.pnas.org/cgi/doi/10.1073/pnas.0901404106.

Bali R., L. Savino, D.A. Ramirez, N.M. Tsvetkova, L. Bagatolli, F. Tablin, J.H. Crowe, C. Leidy- 2009-Macroscopic domain formation during cooling in the platelet plasma membrane: An issue of low cholesterol content- Biochimica et Biophysica Acta 1788: 1229-1237, doi: 10.1016/j.bbamem.2009.03.017.

Balycheva M., G. Faggian, A.V. Glukhov, J. Gorelik- 2015-Microdomain-specific localization of functional ion channels in cardiomyocytes: an emerging concept of local regulation and remodeling- Biophysical Reviews 7: 43-62, doi: 10.1007/s12551-014-0159-x.

Banigan J.R., M. Leninger, A.S. Her, N.J. Traaseth- 2018-Assessing interactions between a polytopic membrane protein and lipid bilayers using differential scanning calorimetry and solid-state NMR- Journal of Physical Chemistry B 122: (#8) 2314-2322, doi: 10.1021/asc.jpcb.8b00479.

Bean B.P- 2007-The action potential in mammalian central neurons- Nature Reviews Neuroscience 8: (June) 451-465, doi:10.1038/nrn2148.

Beilby M.J- 2007-Action potential in Charophytes- International Review of Cytology 257: 43-82, doi: 10.1016/S0074-7696(07)57002-6.

Beker B.M., C. Cervellera, A. De Vito, C.G. Musso- 2018-Human physiology in extreme heat and cold- International Archives of Clinical Physiology 1: (#1) 001, 8 pgs.

Bélanger M., I. Allaman, P.J. Magistretti- 2011-Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation- Cell Metabolism 14: (12/7) 724-738, doi: 10.1016/j.cmet.2011.08.016.

Belosludtsev K.N., N.V. Belosludtseva, A.V. Agafonov, N.V. Penkov, V.N. Samartsev, J.J. Lemasters, G.D. Mironova- 2015-Effect of surface-potential modulators on the opening of lipid pores in liposomal and mitochondrial inner membranes induced by palmitate and calcium ions- Biochimica et Biophysica Acta 1848: 2200-2205, http://dx.doi.org/10.1016/j.bbamem.2015.05.013.

Benjwal S., O. Gursky- 2010-Pressure perturbation calorimetry of apolipoproteins in solution and in model lipoproteins- Proteins 78: (#5) 1175-1185, doi: 10.1002/prot.22637.

Berg R.W., M.T. Stauning, J.B. Sørensen, H. Jahnsen- 2017-Comment on "Penetration of action potentials during collision in the median and lateral giant axons of invertebrates"- Physical Review X 7: 028001, 3 pgs. doi: 10.1103/PhysRevX.7.028001.

Bezanilla F- 2006-The action potential: From voltage-gated conductances to molecular structures- Biological Research 39: 425-435.

Bhattacharya S., S. Haldar- 2000-Interactions between cholesterol and lipids in bilayer membranes. Role of lipid headgroup and hydrocarbon chain-backbone linkage- Biochimica et Biophysica Acta 1467: 39-53.

Bianchi F., M. Molboubi, J.H. George, A. Jerusalem, M.S. Thompson, H. Ye- 2019-Ion current and action potential alterations in peripheral neurons subject to uniaxial strain- Journal of Neuroscience Research 97: 744-751, doi: 10.1002/jnr.24408.

Birkenhauer E., S. Neethirajan- 2014-Surface potential measurement of bacteria using Kelvin Probe Force Microscopy- Journal of Visualized Experiments 93: 8 pgs, e52327, doi: 10.3791/52327.

Bolton C.F., G.M. Sawa, K. Carter- 1981-The effects of temperature on human compound action potentials- Journal of Neurology, Neurosurgery, and Psychiatry 44: 407-413.

Bonincontro A., K.H. Nierhaus, G. Onori, G. Risuleo- 2001-Intrinsic structural differences between ‘tight couples’ and Kaltschmidt-Wittmann ribosomes evidenced by dielectric spectroscopy and scanning microcalorimetry- FEBS Letters 490: 93-96.

Brauchi S., P. Orio, R. Latorre- 2004-Clued to understanding cold sensation: Thermodynamics and electrophysiological analysis of the cold receptor TRPM8- Proceedings of the National Academy of Sciences U.S.A. 101: (#43) 15494-15499, www.pnas.org/cgi/doi/10.1073/pnas.0406773101.

Brown D.A., E. London- 1998-Functions of lipid rafts in biological membranes- Annual Review of Cell and Developmental Biology 14: 111-136.

Buehler L.K- 2016-Cell Membranes- 382 pgs. Garland Science. New York, New York, U.S.A.

Canac N., K. Jalaleddini, S.G. Thorpe, C.M. Thibeault, R.B. Hamilton- 2020-Review: pathophysiology of intracranial hypertension and noninvasive intracranial pressure monitoring- Fluids and Barriers of the CNS 17: 40, 21 pgs., https://doi.org/10.1186/s12987-020-00201-8.

Cañadas O., I. García-Verdugo, K.M.W. Keough, C. Casals- 2008-SP-A permeabilizes lipopolysaccharide membranes by forming protein aggregates that extract lipids from the membrane- Biophysical Journal 95: (Oct.) 3287-3294, doi: 10.1529/biophysj.108.137323.

Cario A., V. Grossi, P. Schaeffer, P.M. Oger- 2015-Membrane homeoviscous adaptation in the piezo-hyperthermophilic archaeon *Thermococcus barophilus*- Frontiers in Microbiology 6: 1152, doi: 10.3389/fmicb.2015.01152.

Carrillo N., T. Knuuttila- 2022-Holistic idealization: An artifactual standpoint- Studies in History and Philosophy of Science 91: 49-59, https://doi.org/10.1016/j.shpsa.2021.10.009.

Cavanagh J.F- 2018-Electrophysiology as a theoretical and methodological hub for the neural sciences- Psychophysiology 56: e13314, 13 pgs., doi: 10.1111/psyp.13314.

Chen J.C.T., M. Chesler- 1991-Extracellular alkalinization evoked by GABA and its relationship to activity-dependent pH shifts in turtle cerebellum- Journal of Physiology 442: 431-446.

Chen S., H. Dong, J. Yang- 2020-Surface potential/charge sensing techniques and applications- Sensors 20: 1690, 18 pgs., doi: 10.3390/s20061690.

Chesler M- 2003-Regulation and modulation of pH in the brain- Physiological Reviews 83: 1183-1221, doi: 10.1152/physrev.00010.2003.

Chiu S.Y., J.M. Ritchie- 1982-Evidence for the presence of potassium channels in the internode of frog myelinated nerve fibres- Journal of Physiology 322: 485-501.

Chong P.L-G., U. Ayesa, V.P. Daswani, E.C. Hur- 2012-On physical properties of tetraether lipid membranes: Effects of cyclopentane rings- Archaea article 138439, 11 pgs., doi: 10.1155/2012/138439.

Chong P.L-G., M. Sulc, R. Winter- 2010-Compressibilities and volume fluctuations of Archaeal tetraether liposomes- Biophysical Journal 99: (Nov.) 3319-3326, doi: 10.1016/j.bpj.2010.09.061.

Chugunov A.O., P.E. Volynsky, N.A. Krylov, I.A. Boldyrev, R.G. Efremov- 2014-Liquid but durable: Molecular dynamics simulations explain the unique properties of Archaeal-like membranes- Scientific Reports 4: 7462, 8 pgs, doi: 10.1038/srep07462.

Cleland C.E- 2001-Historical science, experimental science, and the scientific method- Geology 29: (#11, Nov.) 987-990.

Cohen C.C.H., M.A. Popovic, J. Klooster, M-T. Weil, W. Möbius, K-A. Nave, M.H.P. Kole- 2020-Saltatory conduction along myelinated axons involves a periaxonal nanocircuit- Cell 180: 311-322, https://doi.org/10.1016/j.cell.2019.11.039.

Cohen L.B., B. Hille, R.D. Keynes, D. Landowne, E. Rojas- 1971-Analysis of the potential-dependent changes in optical retardation in the squid giant axon- Journal of Physiology 218: 205-237.

Cornejo V.H., N. Ofer, R. Yuste- 2022-Voltage compartmentalization in dendritic spines in vivo- Science 375: (#6576, 1/7) 82-86, doi: 10.1126/science.abg0501.

Counillon L., Y. Bouret, I. Marchiq, J. Pouysségur- 2016-Na^+/H^+ antiporter (NHE1) and lactate/H^+ symporters (MCTs) in pH homeostasis and cancer metabolism- Biochimica et Biophysica Acta 1863: 2465-2480, http://dx.doi.org/10.1016/j.bbamcr.2016.02.018.

Crampin E.J., N.P. Smith, A.E. Langham, R.H. Clayton, C.H. Orchard- 2006-Acidosis in models of cardiac ventricular myocytes- Philosophical Transactions of the Royal Society A 364: 1171-1186, doi: 10.1098/rsta.2006.1763.

Crivellin A., M. Hoferichter- 2021-Hints of lepton flavor universality violations- Science 374: (#6571, 11/26) 1051-1052, DOI: 10.1126/science.abk2450.

Crotty P., T. Sangrey, W.B. Levy- 2006-Metabolic energy cost of action potential velocity- Journal of Neurophysiology 96: 1237-1246, doi: 10.1152/jn.01204.2005.

Crowe J.H., F.A. Hoekstra, L.M. Crowe, T.J. Anchordoguy, E. Drobnis- 1989-Lipid phase transitions measured in intact cells with Fourier Tranform infrared spectroscopy- Cryobiology 26: 76-84.

Darwin C- 1889-The Descent of Man, and Selection in Relation to Sex. 2nd edition. 688 pgs. Appleton and Company. New York, New York, U.S.A.

Das P., W.H. Schwarz- 1995-Solitons in cell membranes- Physical Review E 51: (#4) 3588-3612, doi: 10.1103/PhysRevE.51.3588.

Delahay P- 1966-Sturcture of the diffusion double layer in the absence of specific adsorption- Chapter 3, pgs. 33-52, in Double layer and electrode kinetics. Interscience Publishers. New York, New York, U.S.A.

de Lera Ruiz M., R.L. Kraus- 2015-Voltage-gated sodium channels: Structure, function, pharmacology, and clinical indications- Journal of Medicinal Chemistry 58: 7093-7118, doi: 10.1021/jm.501981g.

de Lichtervelde A.C.L., J.P. de Souza, M.Z. Bazant- 2020-Heat of nervous conduction: A thermodynamic framework- Physical Review E 101: 022406, 13 pgs, doi: 10.1103/PhysRevE.101.022406.

den Hertog A., P. Greengard, J.M. Ritchie- 1969-On the metabolic basis of nervous activity- Journal of Physiology 204: 511-521.

Drouin H., R. The- 1969-The effect of reducing extracellular pH on the membrane currents of the Ranvier node- Pflügers Arch. 313: 80-88.

Drukarch B., M.M.M. Wilhelmus, S. Shrivastava- 2021-The thermodynamic theory of action potential propagation: a sound basis for unification of the physics of nerve impulses- Reviews in the Neurosciences 18 pgs, https://doi.org/10.1515/revneuro-2021-0094.

Ebel H., P. Grabitz, T. Heimburg- 2001-Enthalpy and volume changes in lipid membranes. I. The proportionality of heat and volume changes in the lipid melting transition and its implication for the elastic constants- Journal of Physical Chemistry B 105: 7353-7360, doi: 10.1021/jp010515s

Eisenberg S., E. Haimov, G.F.W. Walpole, J. Plumb, M.M. Kozlov, S. Grinstein- 2021-Mapping the electrostatic profiles of cellular membranes- Molecular Biology of the Cell 32: (Feb. 1) 301-310, doi: 10.1091/mbc.E19-08-0436.

El Hady A., B.B. Machta- 2015-Mechanical surface waves accompany action potential propagation- Nature Communications 6: 6697, 7 pgs., doi: 10.1038/ncomms7697.

Fabiunke S., C. Fillafer, A. Paeger, M.F. Schneider- 2021-Optical studies of membrane state during action potential propagation- Progress in Biophysics and Molecular Biology 162: 69-78, https://doi.org/10.1016/j.pbiomolbio.2020.11.001.

Fedosejevs C.S., M.F. Schneider- 2022-Sharp, localized phase transitions in single neuronal cells- Proceedings of the National Academy of Sciences U.S.A. 119: (#8) e2117521119, 6 pgs., https://doi.org/10.1073/pnas.2117521119.

Feigenson G.W- 2007-Phase boundaries and biological membranes- Annual Review of Biophysics and Biomolecular Structure 36: 63-77, doi: 10.1146/annurev.biophys.36.040306.132721.

Fernández J.M., R.E. Taylor, F. Bezanilla- 1983-Induced capacitance in the squid giant axon- The Journal of General Physiology 83: (Sept.) 331-346.

Fichtl B., I. Silman, M.F. Schneider- 2018-On the physical basis of biological signaling by interface pulses- Langmuir 34: 4914-4919, DOI: 10.1021/acs.langmuir.7b01613.

Fichtl B., S. Shrivastava, M.F. Schneider- 2016-Protons at the speed of sound: Predicting specific biological signaling from physics- Scientific Reports 6: 22874, 9 pgs, doi: 10.1038/srep22874.

Fidorra M., T. Heimburg, LA. Bagatolli- 2009-Direct visualization of the lateral structure of porcine brain cerebrosides/POPC mixtures in presence and absence of cholesterol- Biophysical Journal 97: (July) 142-154, doi: 10.1016/j.bpj.2009.03.060.

Fillafer C., Y.S. Koll, M.F. Schneider- 2022-Lipid membrane state change by catalytic protonation and the implications for synaptic transmission- Membranes 12: (#5) 16 pgs, https://doi.org/10.3390/membranes12010005.

Fillafer C., A. Paeger, M.F. Schneider- 2021-The living state: How cellular excitability is controlled by thermodynamic state of the membrane- Progress in Biophysics and Molecular Biology 162: 57-68, https://doi.org/10.1016/j.pbiomolbio.2020.10.003.

Fillafer C., M. Mussel, J. Muchowski, M.F. Schneider- 2018-On cell surface deformation during an action potential- Biophysical Journal 114: (#2) 410-418, doi: 10.1016/j.bpj.2017.11.3776.
Fillafer C., A. Paeger, M.F. Schneider- 2017-Collision of two action potentials in a single excitable cell- Biochemical et Biophysica Acta 1861: 3282-3286, http://dx.doi.org/10.1016/j.bbagen.2017.09.020.

Fillafer C., M.F. Schneider- 2016-On the excitation of action potentials by protons and its potential implications for cholinergic transmission- Protoplasma 253: 357-365, doi: 10.1007/s00709-015-0815-4.

Fillafer C., M.F. Schneider- 2013-On the temperature behavior of pulse propagation and relaxation in worms, nerves and gels- PLoS One 8: (#6) e66773, 6 pgs, doi: 10.1371/journal.pone.0066773.

Fliegel L- 2019-Structural and functional changes in the Na^+/H^+ exchanger isoform 1, induced by Erk1/2 phosphorylation- International Journal of Molecular Sciences 20: 2378, 19 pgs., doi: 10.3390/ijms20102378.

Foster V.S., L.D. Rash, G.F. King, M.M. Rank- 2021-Acid-sensing ion channels: Expression and function in resident and infiltrating immune cells in the central nervous system- Frontiers in Cellular Neuroscience 15: 738043, 16 pgs., doi: 10.3389/fncel.2021.738043.

Fozzard H.A- 1966-Membrane capacity of the cardiac Purkinje fibre- Journal of Physiology 182: 255-267.

Franco I., E. Castillo, M-D. Pérez, M. Calvo, L. Sánchez- 2012-Effects of hydrostatic high pressure on the structure and antibacterial activity of recombinant human lactoferrin from transgenic rice- Bioscience, Biotechnology and Biochemistry 76: (#1) 53-59, doi: 10.1271/bbb.110433.

Franz D.N., A. Iggo- 1968-Conduction failure in myelinated and nonmyelinated axons at low temperatures- Journal of Physiology 199: 319-345.

Franzen D.L., S.A. Gleiss, C. Berger, F.S. Kümpfbeck, J.J. Ammer, F. Felmy- 2015-Development and modulation of intrinsic membrane properties control the temporal precision of auditory brain stem neurons- Journal of Neurophysiology 113: 524-536, doi: 10.1151/jn.00601.2014.

Fribrance S., J. Wang, J.R. Roppolo, W.C. de Groat, C. Tai- 2016-Axonal model for temperature stimulation- Journal of Computational Neuroscience 41: 185-192, doi: 10.1007/s10827-016-0612-x.

Fromm J., S. Lautner- 2007-Electrical signals and their physiological signifiance in plants- Plant, Cell and Environment 30: 249-257, doi: 10.1111/j.1365-3040.2006.01614x.

Fudge D.S- 2014-Fifty years of J. R. Platt's strong inference- The Journal of Experimental Biology 217: 1202-1204, doi: 10.1242/jeb.104976.

Galassi V.V., N. Wilke- 2021-On the coupling between mechanical properties and electrostatics in biological membranes- Membranes 11: 478, 24 pgs., https://doi.org/10.3390/membranes11070478.

Gautier J., S. Passot, C. Pénicaud, H. Guillemin, S. Cenard, P. Lieben, F. Fonseca- 2013-A low membrane lipid phase transition temperature is associated with a high cryotolerance of *Lactobacillus delbrueckii* subspecies *bulgaricus* CFL1- Journal of Dairy Science 96: 5591-5602, http://dx.doi.org/10.3168/jds.2013-6802.

Genz A., F.J. Holzwarth, T.Y. Tsong- 1986-The influence of cholesterol on the main phase transition of unilamellar dipalmytoylphosphatidylcholine vesicles. A differential scanning calorimetry and iodine laser T-jump study- Biophysical Journal 50: (Dec.) 1043-1051.

Gill P., T.T. Moghadam, B. Ranjbar- 2010-Differential scanning calorimetry techniques: Applications in biology and nanoscience- Journal of Biomolecular Techniques 21: (#4) 167-193.

Glase J.C- 2002-Biology as a science- Chapter 2, pgs. 25-32, in Investigative Biology: A Laboratory Text, 2002-2003, BIO 103-104. J. C. Glase and P.R. Ecklund editors. Cornell University. Ithaca, New York, U.S.A.

Gluick T.C., D.E. Draper- 1994-Thermodynamics of folding a pseudoknotted mRNA fragment- Journal of Molecular Biology 241: 246-262.

Golod D.A., R. Kumar, R.W. Joyner- 1998-Determinants of action potential initiation in isolated rabbit atrial and ventricular myocytes- American Journal of Physiology 274: (#6, part 2, June) H1902-H1913.

Gonzalez-Perez A., L.D. Mosgaard, R. Budvytyte, E. Villagran-Vargas, A.D. Jackson, T. Heimburg- 2016-Solitary electromechanical pulses in lobster neurons- Biophysical Chemistry 216: 51-59, http://dx.doi.org/10.1016/j.bpc.2016.06.005.

Gonzalez-Perez A., R. Budvytyte, L.D. Mosgaard, S. Nissen, T. Heimburg- 2014-Penetration of action potentials during collision in the median and lateral giant axons of invertebrates- Physical review X 4: 031047, 12 pgs, doi: 10.1103/PhysRevX.4.031047.

Grabitz P., V.P. Ivanova, T. Heimburg- 2002-Relaxation kinetics of lipid membranes and its relation to the heat capacity- Biophysical Journal 82: (Jan.) 299-309.

Greffrath W., S.T. Schwarz, D. Büsselberg, R-D. Treede- 2009-Heat-induced action potential discharges in nociceptive primary sensory neurons of rats- Journal of Neurophysiology 102: 424-436, doi: 10.1152/jn.90916.2008.

Griesbauer J., S. Bössinger, A. Wixforth, M.F. Schneider- 2012-Simultaneously propagating voltage and pressure pulses in lipid monolayers of pork brain and synthetic lipids- Physical Review E 86: 061909, 11 pgs, DOI: 10.1103/PhysRevE.86.061909.

Griesbauer J., A. Wixforth, M.F. Schneider- 2009-Wave propagation in lipid monolayers- Biophysical Journal 97: (Nov.) 2710-2716, doi: 10.1016/j.bpj.2009.07.049.

Gudmand M., M. Fidorra, T. Bjørnholm, T. Heimburg- 2009-Diffusion and partitioning of fluorescent lipid probes in phospholipid monolayers- Biophysical Journal 96: (June) 4598-4609, doi: 10.1016/j.bpj.2009.01.063.

Gulledge A.T., J.J. Bravo- 2016-Neuron morphology influences axon initial segment plasticity- eNeuro 3: (#1, Jan/Feb) 1-24, e0085-15.2016, http://dx.doi.org/10.1523/ENEURO.0085-15.2016.

Gutsmann T., T. Heimburg, U. Keyser, K.R. Mahendran, M. Winterhalter- 2015-Protein reconstitution into freestanding planar lipid membranes for electrophysiological characterization- Nature Protocols 10: (#1) 188-198, doi: 10.1038/nprot.2015.003.

Hac A.E., H.M. Seeger, M. Fidorra, T. Heimburg- 2005-Diffusion in two-component lipid membranes - A fluorescence correlation spectroscopy and Monte Carlo simulation study- Biophysical Journal 88: (Jan.) 317-333, doi: 10.1529/biophysj.104.040444.

Halstenberg S., T. Heimburg, T. Hianik, U. Kaatze, R. Krivanek- 1998-Cholesterol-induced variations in the volume and enthalpy fluctuations of lipid bilayers- Biophysical Journal 75: (July) 264-271.

Harary M., R.G.F. Dolmans, W.B. Gormley- 2018-Intracranial pressure monitoring - Review and avenues for development- Sensors 18: 465, 15 pgs., doi: 10.3390/s18020465.

Hayashi H., O. Aharonovitz, R.T. Alexander, N. Touret, W. Furuya, J. Orlowski, S. Grinstein- 2008-Na^+/H^+ exchange and pH regulation in the control of neutrophil chemokinesis and chemotaxis- American Journal of Physiology and Cell Physiology 294: C526-C534, doi: 10/1152/ajpcell.00219.2007.

Haynie D.T- 2008-Biological Thermodynamics, 2nd edition. 422 pgs. Cambridge University Press, Cambridge, U.K.

Hays L.M., R.E. Feeney, L.M. Crowe, J.H. Crowe, A.E. Oliver- 1996-Antifreeze glycoproteins inhibit leakage from liposomes during thermotropic phase transitions- Proceedings of the National Academy of Sciences U.S.A. 93: 6835-6840.

He C., X. Hu, R.S. Jung, T.A. Weston, N.P. Sandoval, P. Tontonoz, M.R. Kilburn, L.G. Fong, S.G. Young, H. Jiang- 2017-High-resolution imaging and quantification of plasma membrane cholesterol by NanoSIMS- Proceedings of the National Academy of Sciences U.S.A. 114: (#8) 2000-2005, www.pnas.org/cgi/doi/10.1073/pnas.1621432114.

Heimburg T- 2021-The important consequences of the reversible heat production in nerves and the abiabaticity of the action potential- Progress in Biophysics and Molecular Biology 162: 26-40. https://doi.org/10.1016/j.pbiomolbio.2020.07.007.

Heimburg T- 2019-Phase transitions in biological membranes- Pgs. 39-61, in, Thermodynamics and Biophysics of Biomedical Nanosystems. C. Demetzos and N. Pippa editors. Series in BioEngineering, Springer Nature, Singapore. doi: 10.1007/978-981-13-0989-2_3.

Heimburg T- 2018-Comment on Tamagawa and Ikeda's reinterpretation of the Goldman-Hodgkin-Katz equation- European Biophysics Journal 47: 865-867, https://doi.org/10.1007/s00249-018-1335-x.

Heimburg T- 2012-The capacitance and electromechanical coupling of lipid membranes close to transitions: The effect of electrostriction- Biophysical Journal 103: (Sept.) 918-929, http://dx.doi.org/10.1016/j.bpj.2012.07.010.

Heimburg T- 2010-Lipid ion channels- Biophysical Chemistry 150: 2-22, doi: 10.1016/j.bpc.2010.02.018.

Heimburg T- 2009-The physics of nerves (English Translation)- Physik Journal 8: (#3) 33-39.

Heimburg T- 2007-Thermal Biophysics of Membranes- Tutorials in Biophysics- 363 pgs. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany.

Heimburg T- 2000-A model for the lipid pretransition: Coupling of ripple formation with the chain-melting transition- Biophysical Journal 78: (Mar.) 1154-1165.

Heimburg T- 1998-Mechanical aspects of membrane thermodynamics. Estimation of the mechanical properties of lipid membranes close to the chain melting transition from calorimetry- Biochimica et Biophysica Acta 1415: 147-162.

Heimburg T., B. Angerstein, D. Marsh- 1999-Binding of peripheral proteins to mixed lipid membranes: Effect of lipid demixing upon binding- Biophysical Journal 76: (May) 2575-2586.

Heimburg T., R.L. Biltonen- 1996-A Monte Carlo simulation study of protein-induced heat capacity changes and lipid-induced protein clustering- Biophysical Journal 70: (Jan.) 84-96.

Heimburg T., R.L. Biltonen- 1994-Thermotropic behavior of dimyristoylphosphatidylglycerol and its interaction with cytochrome *c*- Biochemistry 33: 9477-9488.

Heimburg T., P. Hildebrandt, D. Marsh- 1991-Cytochome *c*-lipid interactions studied by resonance Raman and ^{31}P NMR spectroscopy. Correlation between the conformational changes of the protein and the lipid bilayer- Biochemistry 30: 9084-9089.

Heimburg T., A.D. Jackson- 2007-The thermodynamics of general anesthesia- Biophysical Journal 92: (May) 3159-3165, doi: 10.1529/biophysj.106.099754.

Heimburg T., A.D. Jackson- 2006-On the action potential as a propagating density pulse and the role of anesthetics- arXiv:physics/ob10117v2 [physics.bio-ph] 13 pgs.

 doi:10.20944/preprints202208.0248.v1

Heimburg T., A.D. Jackson- 2005-On soliton propagation in biomembranes and nerves- Proceeding of the National Academy of Sciences U.S.A. 102: (#28, July 12) 9790-9795, www.pnas.org/cgi/doi/10.1073/pnas.0503823102.

Heimburg T., D. Marsh- 1993-Investigation of secondary and tertiary structural changes of cytochrome *c* in complexes with anionic lipids using amide hydrogen exchange measurements: An FTIR study- Biophysical Journal 65: (Dec.) 2408-2417.

Heimburg T., U. Würz, D. Marsh- 1992-Binary phase diagram of hydrated dimyristoylglycerol-dimyristoylphosphatidylcholine mixtures- Biophysical Journal 63: (Nov.) 1369-1378.

Henze D.A., G. Buzsáki- 2001-Action potential threshold of hippocampal pyramidal cell *in vivo* is increased by recent spiking activity- Neuroscience 105: (#1) 121-130.

Heus H.A., J.M.A. Van Kimmenade, P.H. van Knippenberg, H-J. Hinz- 1983-Calorimetric measurements of the destabilization of a ribosomal RNA hairpin by dimethylation of two adjacent adenosines- Nucleic Acids Research 11: (#1) 203-210.

Hildebrandt P., T. Heimburg, D. Marsh, G.L. Powell- 1990-Conformational changes in cytochrome *c* and cytochrome oxidase upon complex formation: A resonance raman study- Biochemistry 29: 1661-1668.

Hille B- 1984-Ionic Channels of Excitable Membranes- 426 pgs. Sinauer Assoc., Inc. Sunderland, Mass., U.S.A.

Hille B- 1968-Charges and potentials at the nerve surface. Divalent ions and pH- Journal of General Physiology 51: 221-236.

Hodgkin A.L- 1937a-Evidence for electrical transmission in nerve. Part I- Journal of Physiology 90: 183-210.

Hodgkin A.L- 1937b-Evidence for electrical transmission in nerve. Part II- Journal of Physiology 90: 211-232.

Hodgkin A.L., A.F. Huxley- 1939-Action potentials recorded from inside a nerve fibre- Nature 144: (Oct. 21) 710-711.

Hodgkin A.L., A.F. Huxley - 1945-Resting and action potentials in single nerve fibres- Journal of Physiology 104: 176-195.

Hodgkin A.L., A.F. Huxley- 1952a-A quantitative description of membrane current and its application to conduction and excitation in nerve- Journal of Physiology 117: 500-544.

Hodgkin A.L., A.F. Huxley- 1952b-Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*- Journal of Physiology 116: 449-472.

Hodgkin A.L., A.F. Huxley- 1952c-The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*- Journal of Physiology 116: 497-506.

Hodgkin A.L., A.F. Huxley- 1952d-The components of membrane conductance in the giant axon of *Loligo*- Journal of Physiology 116: 473-496.

Hodgkin A.L., A.F. Huxley, B. Katz- 1952e-Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*- Journal of Physiology 116: 424-448.

Hodgkin A.L., B. Katz- 1949-The effect of sodium ions on the electrical activity of the giant axon of the squid- Journal of Physiology 108: 37-77.

Hodgkin A.L., R.D. Keynes- 1955-The potassium permeability of a giant nerve fibre- Journal of Physiology 128: 61-88.

Holzenburg A., P.C. Jones, T. Franklin, T. Pali, T. Heimburg, D. Marsh, J.B.C. Findlay, M.E. Finbow- 1993-Evidence for a common structure for a class of membrane channels- European Journal of Biochemistry 213: 21-30.

Homann U., G. Thiel- 1994-Cl^- and K^+ channel currents during the action potential in *Chara*. Simultaneous recording of membrane voltage and patch currents- Journal of Membrane Biology 141: 297-309.

Honigmann A., V. Mueller, H. Ta, A. Schoenle, E. Sezgin, S.W. Hell, C. Eggeling- 2014-Scanning STED-FCS reveals spatiotemporal heterogeneity of lipid interaction in the plasma membrane of living cells- Nature Communications 5: 5412, 12 pgs., doi: 10.1038/ncomms6412.

Howarth J.V., R.D. Keynes, J.M. Ritchie- 1968-The origin of the initial heat associated with a single impulse in mammalian non-myelinated nerve fibres- Journal of Physiology 194: 745-793.

Huang R-Q., G.H. Dillon- 1999-Effect of extracellular pH on GABA-activated current in rate recombinant receptors and thin hypothalamic slices- Journal of Neurophysiology 82: (#3) 1233-1243.

Huxley A- 2002a-From overshoot to voltage clamp- Trends in Neuroscience 25: (#11) 553-558.

Huxley A.F- 2002b-Hodgkin and the action potential 1935-1952- Journal of Physiology 538: (#1) 2, doi: 10.1013/jphysiol.2001.014118.

Inoue I., Y. Kobatake, I. Tasaki- 1973-Excitability, instability and phase transitions in squid axon membrane under internal perfusion with dilute salt solutions- Biochimica et Biophysica Acta 307: 471-477.

Iosub R., D. Avitabile, L. Grant, K. Tsaneva-Atanasova, H.J. Kennedy- 2015-Calcium-induced calcium release during action potential firing in developing inner hair cells- Biophysical Journal 108: (March) 1003-1012, http://dx.doi.org/10.1016/j.bpj.2014.11.3489.

Ivanova V.P., T. Heimburg- 2001-Histogram method to obtain heat capacities in lipid monolayers, curved bilayers, and membranes containing peptides- Physical Review E 63: 041914, 12 pgs. doi: 10.1103/PhysRevE.63.041914.

Jackson A.D., T. Heimburg- 2020-Comment "On biological signaling" by G. Nimtz and H. Aichmann, Z. Naturforsch. 75a: 507509, 2020- Physics ArXiv, physics.bio-ph, arXiv:2008.07266v2, 24 Aug 2020, 2 pgs.

Jang I-S., M. Nakamura, H. Kubota, M. Noda, N. Akaike- 2020-Extracellular pH modulation of excitatory synaptic transmission in hippocampal CA3 neurons- Journal of Neurophysiology 123: 2426-2436, doi: 10.1152/jn.00013.2020.

Jayaraman S., R. Jasuja, M.N. Zakharov, O. Gursky- 2011-Pressure perturbation calorimetry of lipoproteins reveals an endothermic transition without detectable volume changes. Implications for adsorption of apolipoprotein to a phospholipid surface- Biochemistry 50: 3919-3927, dx.doi.org/10.1021/bi200090y.

Jensen R., J. Nielsen, N. Ørtenbald- 2020-Inhibition of glycogenolysis prolongs action potential repriming period and impairs muscle function in rat skeletal muscle- Journal of Physiology 598: (#4) 798-803, doi: 10.1113/JP278543.

Jerusalem A., Z. Al-Rekabi, H. Chen, A. Ercole, M. Malboubi, M. Tamayo-Elizalde, L. Verhagen, S. Contera- 2019-Electrophysiological-mechanical coupling in the neuronal membrane and its role in ultrasound neuromodulation and general anaesthesia- Acta Biomaterialia 97: 116-140, https://doi.org/10.1016/j.actbio.2019.07.041.

Jin L., A.C. Millard, J.P. Wuskell, H.A. Clark, L.M. Loew- 2005-Cholesterol-enriched lipid domains can be visualized by di-4-ANEPPDHQ with linear and nonlinear optics- Biophysical Journal- Biophyical Letters, L04-L06, doi: 10.1529/biophysj.105.064816.

Jin L., A.C. Millard, J.P. Wuskell, X. Dong, D. Wu, H.A. Clark, L.M. Loew- 2006- Characterization and application of a new optical probe for membrane lipid domains- Biophysical Journal 90: (April) 2563-2575, doi: 10.1529/biophysj.105.072884.

Joersbo M., J. Brunstedt, F. Floto- 1990-Quantitative relationship between parameters of electroporation- Journal of Plant Physiology 137: 169-174.

Johnson B.R., R.A. Wyttenbach, R. Wayne, R.R. Hoy- 2002-Action potentials in a giant algal cell: A comparative approach to mechanisms and evolution of excitability- The Journal of Undergraduate Neuroscience Education 1: (#1) A23-A27.

Jones D.K, C.H. Peters, S.A. Tolhurst, T.W. Claydon, P.C. Ruben- 2011-Extracellular proton modulation of the cardiac voltage-gated sodium channel, $Na_v1.5$- Biophysical Journal 101: (Nov.) 2147-2156, doi: 10.1016/j.bpj.2011.08.056.

Jones M.N., D. Chapman- 1995-Micelles, Monolayers, and Biomembranes- 252 pgs., Wiley-Liss, N.Y., U.S.A.

Julian F.J., D.E. Goldman- 1962-The effects of mechanical stimulation on some electrical properties of axons- Journal of General Physiology 46: 297-313.

Jurisic N- 1987-The propagation of the nerve impulse- Biophysical Journal 51: (May) 817-823.

Käkelä R., M. Mattila, M. Hermansson, P. Haimi, A. Uphoff, V. Paajanen, P. Somerharju, M. Vornanen- 2008-Seasonal acclimatization of brain lipidome in a eurythermal fish (*Carrassius carassius*) is mainly determined by temperature- American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 294: R1716-R1728, doi:10.1152/ajpregu.00883.2007.

Kaletunç G., J. Lee, H. Alpas, F. Bozoglu- 2004-Evaluation of structural changes induced by high hydrostatic pressure in *Leuconostoc mesenteroides*- Applied and Environmental Microbiology 70: (#2) 1116-1122, doi: 10.1128/AEM.70.2.1116-1122.2004.

Kang K.H., M.F. Schneider- 2020-Nonlinear pulses at the interface and its relation to state and temperature- European Physical Journal E 48: (#8) 5 pgs., doi: 10/1140/epje/i2020-11903-x.

Kann O- 2016-The interneuron energy hypothesis: Implications for brain disease- Neurobiology of Disease 90: 75-85, http://dx.doi.org/10.1016/j.nbd.2015.08.005.

Kapoor S., A. Werkmüller, C. Denter, Y. Zhai, J. Markgraf, K. Weise, N. Opitz, R. Winter- 2011-Temperature-pressure phase diagram of a heterogeneous anionic model biomembrane system: Results from a combined calorimetry, spectroscopy and microscopy study- Biochimica et Biophysica Acta 1808: 1187-1195, doi: 10.1016/j.bbamem.2011.01.011.

Kappler J., S. Shrivastava, M.F. Schneider, R.R. Netz- 2017-Nonlinear fractional waves at elastic interfaces- Physics arXiv: 1702.08864v1 [physics.flu-dyn] 28 Feb 2017, 12 pgs., (Published in Physical Review Fluids, Oct. 2017, doi: 10.1103/PhysRevFluids.2.114804).

Kashirina A.S., I. López-Duarte, M. Kubánková, A.A. Gulin, V.V. Dudenkova, S.A. Rodimova, H.G. Torgomyan, E.V. Zagaynova, A.V. Meleshina, M.K. Kuimova- 2020-Monitoring membrane viscosity in differentiating stem cells using BODIPY-based molecular rotors and FLIM- Scientific Reports 10: 14063, 12 pgs., https://doi.org/10.1038/s41598-020-70972-5.

Keren K- 2011-Cell motility: the integrating role of the plasma membrane- European Biophysical Journal 40: 1013-1027, doi: 10.1007/s00249-011-0741-0.

Keynes R.D., J.M. Ritchie- 1965-The movements of labelled ions in mammalian non-myelinated nerve fibres- Journal of Physiology 179: 333-367.

Kim I., A. Warshel- 2016-A microscopic capacitor model of voltage coupling in membrane proteins: Gating charge fluctuations in Ci-VSD- The Journal of Physical Chemistry B 120: 418-432, doi: 10.1021/asc.jpcb.5b10956.

King A.N., C.F. Manning, J.S. Trimmer- 2014-A unique ion channel clustering domain on the axon initial segment of mammalian neurons- Journal of Computational Neuroscience 522: (#11) 2594-2608, doi: 10.1002/cne.23551.

Kisnieriene V., I. Lapeikaite, V. Pupkis, M.J. Beilby- 2019-Modeling the action potential in Characeae *Nitellopsis obtusa*: Effect of saline stress- Frontiers in Plant Science 10: 82, 10 pgs., doi: 10.3389/fpls.2019.00082.

Kitasato H- 1968-The influence of H^+ on the membrane potential and ion fluxes of *Nitella*- Journal of General Physiology 52: 60-87.

Klumpp D., M. Zimmermann- 1980-Irreversible differential block of A- and C-fibers following local nerve heating in the cat- Journal of Physiology 298: 471-482.

Koga Y- 2012-Thermal adaptation of the Archaeal and Bacterial lipid membranes- Archaea 2012: 789652, 6 pgs., doi: 10.1155/2012/789652.

Kolaric K.V., G. Thomson, J.M. Edgar, A.M. Brown- 2013-Focal axonal swellings and associated ultrastructural changes attenuate conduction velocity in central nervous system axons: a computer modeling study- Physiological Reports 1: (#3) e00059, 19 pgs., doi: 10.1002/phy2.59.

Konyakhina T.M., J. Wu, J.D. Mastroianni, F.A. Heberle, G.W. Feigenson- 2013-Phase diagram of a 4-component lipid mixture: DSPC/DOPC/POPC/chol- Biochimica et Biophysica Acta 1828: 2204-2214, http://dx.doi.org/10.1016/j.bbamem.2013.05.020.

Kosso P- 2011-A summary of Scientific Method- 41 pgs. Springer Press. Heidelberg, Germany. doi: 10.1007/978-94-007-1614-8.

Krupakar J., C.P. Swaminathan, P.K. Das, A. Surolia, S.K. Podder- 1999-Calorimetric studies on the stability of the ribosome-inactivating protein abrin II: effects of pH and ligand binding- Biochemistry Journal 338: 273-279.

Lange Y., S.M.A. Tabei, J. Ye, T.L. Steck- 2013-Stability and stoichiometry of bilayer phospholipid-cholesterol complexes: Relationship to cellular sterol distribution and homeostasis- Biochemistry 52: (#40, Oct. 8), doi: 10.1021/bi400862q.

Laub K.R., K. Witschas, A. Blicher, S.B. Madsen, A. Lückhoff, T. Heimburg- 2012-Comparing ion conductance recordings of synthetic lipid bilayers with cell membranes containing TRP channels- Biochimica et Biophysica Acta- 1818: 1123-1134, doi: 10.1016/j.bbamem.2012.01.014.

Laursen W.J., E.O. Anderson, L.J. Hoffstaetter, S.N. Bagriantsev, E.O. Gracheva- 2015-Species-specific temperature sensitivity of TRPA1- Temperature 2: (#2) 214-226, http://dx.doi.org/10.1080/23328940.2014.1000702.

Lautrup B., R. Appali, A.D. Jackson, T. Heimburg- 2011-The stability of solitons in biomembranes and nerves- The European Physical Journal E 34: 57, 9 pgs, doi: 10.1140/epje/i2011-11057-0.

Lee J., G. Kaletunç- 2002-Evaluation of the heat inactivation of *Escherichia coli* and *Lactobacillus plantarum* by differential scanning calorimetry- Applied and Environmental Microbiology 68: (#11, Nov) 5379-5386, doi: 10.1128/AEM.68.11.5379-5386.2002.

Lee H.J., D. Zhang, Y. Jiang, X. Wu, P-Y. Shih, C-S. Liao, B. Bungart, X-M. Xu, R. Drenan, E. Bartlett, J-X. Cheng- 2017-Label-free vibrational spectroscopic imaging of neuronal membrane potential- Journal of Physical Chemistry Letters- 8: 1932-1936, doi: 10.1021/acs.jpclett.7b00575.

Lehmann P., M. Westberg, P. Tang, L. Lindström, R. Käkelä- 2020-The diapause lipidomes of three closely related beetle species reveal mechanisms for tolerating energetic and cold stress in high-latitude seasonal environments- Frontiers in Physiology 11: (Sept.) 576617, 17 pgs., doi: 10.3389/fphys.2020.576617.

Lei C.L., M. Clerx, K.A. Beattie, D. Melgari, J.C. Hancox, D.J. Gavaghan, L. Polonchuk, K. Wang, G.R. Mirams- 2019-Rapid characterization of hERG channel kinetics II: Temperature dependence- Biophysical Journal 117: (12/17) 2455-2470, doi: 10.1016/j.bpj.2019.07.030.

Leonenko Z., M. Amrein- 2009-The electrical surface potential of pulmonary surfactant- Frontiers in Bioscience 14: (Jan. 1) 4337-4347.

Lewes G.H- 1864-Aristotle: A chapter from the history of science, including analyses of Artistotle's scientific writings- 404 pgs. Smith, Elder and Co. publishers. London, U.K.

Lewis R.N.A.H., R.N. McElhaney- 2013-Membrane lipid phase transitions and phase organization studied by Fourier transform infrared spectroscopy- Biochimica et Biophysica Acta 1828: 2347-2358, http://dx.doi.org/10.1016/j.bbamem.2012.10.018.

Lewis R.N.A.H., R.N. McElhaney- 2000-Calorimetric and spectroscopic studies of the thermotropic phase behavior of lipid bilayer model membranes composed of a homologous series of linear saturated phosphatidylserines- Biophysical Journal 79: (Oct.) 2043-2055.

Lewis R.N.A.H., R.N. McElhaney- 1993a-Calorimetric and spectroscopic studies of the polymorphic phase behavior of a homologous series of n-saturated 1,2-diacyl phosphatidylethanolamines- Biophysical Journal 64: (April) 1081-1096.

Lewis R.N.A.H., R.N. McElhaney- 1993b-Studies of mixed-chain diacyl phosphatidylcholines with highly asymmetric acyl chains: A Fourier transform infrared spectroscopic study of interfacial hydration and hydrocarbon chain packing in the mixed interdigitated gel phase- Biophysical Journal 65: (Nov.) 1866-1877.

Lewis R.N.A.H., D. Zweytick, G. Pabst, K. Lohner, R.N. McElhaney- 2007-Calorimetric, X-ray diffraction, and spectroscopic studies of the thermotropic phase behavior and organization of tetramyristoyl cardiolipin membranes- Biophysical Journal 92: (May) 3166-3177, doi: 10.1529/biophysj.106.094003.

Li S-B., V.M. Damonte, C. Chen, G.X. Wang, J.M. Kebschull, H. Yamaguchi, W-J. Bian, C. Purmann, R. Pattni, A.E. Urban, P. Mourrain, J.A. Kauer, G. Scherrer, L. de Lecea- 2022-Hyperexcitable arousal circuits drive sleep instability during aging- Science 375: (#6583, 2/25) 838, eabh3021, https://doi.org/10.1126/science.abh3021.

Lim C.L., C. Byrne, J.K.W. Lee- 2008-Human thermoregulation and measurement of body temperature in exercise and clinical settings- Annals of the Academy of Medicine, Singapore 37: (#4) 347-353.

Ling T., K.C. Boyle, V. Zuckerman, T. Flores, C. Ramakrishnan, K. Deisseroth, D. Palanker-2020-High-speed interferometric imaging reveals dynamics of neuronal deformation during the action potential- Proceedings of the National Academy of Sciences U.S.A. 117: (#19) 10278-10285, www.pnas.org/cgi/doi/10.1073/pnas.1920039117.

Liu J., H. Tu, D. Zhang, H. Zheng, Y-L. Li- 2012-Voltage-gated sodium channel expression and action potential generation in differentiated NG108-15 cells- BioMed Central Neuroscience 13: 129, 9 pgs., http://www.biomedcentral.com/1471-2202/13/129.

Livingstone C.J., D. Schachter- 1980-Lipid dynamics and lipid-protein interactions in rat hepatocyte plasma membranes- The Journal of Biological Chemistry 255: (#22) 10902-10908.

Lombard J- 2014-Once upon a time the cell membranes: 175 years of cell boundary research- Biology Direct 9: 32, 35 pgs., doi: 10.1186/s13062-014-0032-7.

Lu B-S., S.P. Gupta, M Belička, R. Podgornik, G. Pabst- 2016-Modulation of elasticity and interactions of charged lipid multibilayers: Monovalent salt solutions- Langmuir 32: 13546-13555, doi: 10.1021/acs.langmuir.6b03614.

Lu S.M., G.D. Fairn- 2018-Mesoscale organization of domains in the plasma membrane - beyond the lipid raft- Critical Reviews in Biochemistry and Molecular Biology, 16 pgs., doi: 10.1080/10409238.2018.1436515.

Ma Q.X., A. Arneodo, G.H. Ding, F. Argoul- 2017a-Dynamical study of Na_v channel excitability under mechanical stress- Biological Cybernetics 111: 129-148, doi: 10.1007/s00422-017-0712-3.

Ma Y., A. Benda, J. Kwiatek, D.M. Owen, K. Gaus- 2018-Time-resolved laurdan fluorescence reveals insights into membrane viscosity and hydration levels- Biophysical Journal 115: (Oct.) 1498-1508, https://doi.org/10.1016/j.bpj.2018.08.041.
Ma Y., K. Poole, J. Goyette, K. Gaus- 2017b-Introducing membrane charge and membrane potential to T cell signaling- Frontiers in Immunology 8, 1513, 11 pgs., doi: 10.3389/fimmu.2017.01513.

Magistretti P.J., I. Allaman- 2015-A cellular perspective on brain energy metabolism and functional imaging- Neuron 86: (May 20) 883-901, doi: 10.1016/j.neuron.2015.03.035.

Marsh D- 1996-Lateral pressure in membranes- Biochimica et Biophysica Acta 1286: 183-223.

Martinière A., M. Shvedunova, A.J.W. Thomson, N.H. Evans, S. Penfield, J. Runions, H.G. McWatters- 2011-Homeostasis of plasma membrane viscosity in fluctuating temperatures- New Phytologist- 192: 328-337, doi: 10.1111/j.1469-8137.2011.03821.x.

Mathieu C., R.V. Pappu, J.P. Taylor- 2020-Beyond aggregation: Pathological phase transitions in neurodegenerative disease- Science 370: (#6512, 10/2) 56-60, doi: 10.1126/science.abb8032.

Matsuki H., M. Goto, N. Tamai- 2019-Membrane states of saturated glycerophospholipids: A thermodynamic study of bilayer phase transitions- Chemical Pharmacology Bulletin 67: (#4) 300-307.

Mazurenko S., A. Kunka, K. Beerens, C.M. Johnson, J. Damborsky, Z. Prokop- 2017-Exploration of protein unfolding by modelling calorimetry data from reheating- Scientific Reports 7: 16321, 14 pgs, doi: 10.1038/s41598-017-16360-y.

McClintock P.V.E, I.Kh. Kaufman- 2018-A review of Handbook of Ion Channels, by Jie Zheng and Matthew C. Trudeau- Contemporary Physics 59: (#3) 305-307, doi: 10.1080/00107514.2018.1464063.

McDougal R.A., M.L. Hines, W.W. Lytton- 2013-Reaction-diffusion in the NEURON simulator- Frontiers in Neuroinformatics- 7: 28, 13 pgs., doi: 10.3389/fninf.2013.00028.

McIntyre M.E- 1981-On the 'wave momentum' myth- Journal of Fluid Mechanics 106: 331-347.

McMullen T.P.W., R.N.A.H. Lewis, R.N. McElhaney- 1999-Calorimetric and spectroscopic studies of the effects of cholesterol on the thermotropic phase behavior and organization of a homologous series of linear saturated phsophatidylethanolamine bilayers- Biochimica et Biophysica Acta 1416: 119-134.

Meissner S.T.- 2018-Proposed tests of the soliton wave model of action potentials, and of inducible lipid pores, and how non-electrical phenomena might be consistent with the Hodgkin-Huxley model- Physics arXiv, 45 pgs, arXiv:1808.07193v1 [physics.bio-ph], http://arxiv.org/abs/1808.07193.

Melchior D.L., J.M. Steim- 1976-Thermotropic transitions in biomembranes- Annual Review of Biophysics and Bioengineering 5: 205-238.

Mesquida P., D. Kohl, O.G. Andriotis, P.J. Thurner, M. Duer, S. Bansode, G. Schitter- 2018-Evaluation of surface charge shift of collagen fibrils exposed to glutaraldehyde- Scientific Reports 8: 10126, 7 pgs., doi: 10.1038/s41598-018-28293-1.

Meves H., W. Vogel- 1973-Calcium inward currents in internally perfused giant axons- Journal of Physiology 235: 225-265.

Mills T.T., G.E.S. Toombes, S. Tristram-Nagle, D-M., Smilgies, G.W. Feigenson, J.F. Nagle- 2008-Order parameters and areas in fluid-phase orientated lipid membranes using wide angle X-ray scattering- Biophysical Journal 95: (July) 669-681, doi: 10.1529/biophysj.107.127845.

Mink J.W., R.J. Blumenschine, D.B. Adams- 1981-Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis- American Journal of Physiology 241: R203-R212, https://www.physiology.org/doi/10.1152/ajpregu.1981.241.3.R203.

Money T.G.A., C.I. Rodgers, S.M.K. McGregor, R.M. Robertson- 2009-Loss of potassium homeostasis underlies hyperthermic conduction failure in control and preconditioned locusts- Journal of Neurophysiology 102: 285-293, doi: 10.1152/jn.91174.2008.

Montal M., P. Mueller- 1972-Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties- Proceedings of the National Academy of Sciences U.S.A. 69: (#12, Dec.) 3561-3566.

Morgan D., M. Capasso, B. Musset, V.V. Cherny, E. Rios, M.J.S. Dyer, T.E. DeCoursey- 2009-Voltage-gated proton channels maintain pH in human neutrophils during phagocytosis- Proceedings of the National Academy of Sciences U.S.A. 106: (#42, 10/20) 18022-18027, www.pnas.org/cgi/doi/10.1073/pnas.0905565106.

Mosgaard L.D., K.A. Zecchi, T. Heimburg, R. Budvytyte- 2015a-The effect of the nonlinearity of the response of lipid membranes to voltage perturbations on the interpretation of their electrical properties. A new theoretical description- Membranes 5: 495-512, doi: 10.3390/membranes5040495.

Mosgaard L.D., K.A. Zecchi, T. Heimburg- 2015b-Mechano-capacitive properties of polarized membranes- Soft Matter 11: 7899-7910, doi: 10.1039/c5sm01519g.

Mosgaard L.D., T. Heimburg- 2013-Lipid ion channels and the role of proteins- Accounts of Chemical Research 46: (#12) 2966-2976, doi: 10.1021/ar4000604.

Mouritsen O.G., M.J. Zuckermann- 2004-What's so special about cholesterol?- Lipids 39: (#11) 1101-1113, doi: 10.1007/s11745-004-1336-x.

Muhamed I., F. Crowdhury, V. Maruthamuthu- 2017-Biophysical tools to study cellular mechanotransduction- Bioengineering 4: 12, 27 pgs., doi: 10.3390/bioengineering4010012.

Mussel M., M.F. Schneider- 2021-Sound pulses in lipid membranes and their potential function in biology- Progress in Biophysics and Molecular Biology 162: 101-110, https://doi.org/10.1016/j.pbiomolbio.2020.08.001.

Mussel M., M.F. Schneider- 2019a-It sounds like an action potential: unification of electrical, chemical and mechanical aspects of acoustic pulses in lipids- Journal of the Royal Society Interface 16: 20180743, 10 pgs., http://dx.doi.org/10.1098/rsif.2018.0743.

Mussel M., M.F. Schneider- 2019b-Similarities between action potentials and acoustic pulses in a van der Waals fluid- Scientific Reports 9: 2467, 10 pgs., https://doi.org/10.1038/s41598-019-38826-x.

Mussel M., C. Fillafer, G. Ben-Porath, M.F. Schneider- 2017-Surface deformation during an action potential in pearled cells- Physical Review E 96: 052406, doi: 10.1103/PhysRevE.96.052406.

Mužić T., F. Tounsi, S.B. Madsen, D. Pollakowski, M. Konrad, T. Heimburg- 2019-Melting transitions in biomembranes- Biochemica et Biophysica - Biomembranes 1861: 183026, 11 pgs., doi: 10.1016/j.bbamem.2019.07.014.

Nathan P.W., T.A. Sears- 1962-Differential nerve block by sodium-free and sodium-deficient solutions- Journal of Physiology 164: 375-394.

Nguyen H.T.T., J.E.L. Corry, C.A. Miles- 2006-Heat resistance and mechanism of heat inactivation in thermophilic Campylobacters- Applied and Environmental Microbiology 72: (#1) 908-913, doi: 10.1128/AEM.72.1.908-913.2006.

Nicolini C., J. Kraineva, M. Khurana, N. Periasamy, S.S. Funari, R. Winter- 2006-Temperature and pressure effects on structural and conformational properties of POPC/SM/cholesterol model raft mixtures - a FT-IR, SAXS, DSC, PPC and laurdan fluorescence spectroscopy study- Biochimica et Biophysica Acta 1758: 248-258, doi: 10.1016/j.bbamem.2006.01.019.

Nobel P.S- 1974-Introduction to Biophysical Plant Physiology. 488 pgs. W.H. Freeman and Company. San Francisco, California, U.S.A.

Oh S., C. Fang-Yen, W. Choi, Z. Yaqoob, D. Fu, Y. Park, R.R. Dassari, M.S. Feld- 2012-Label-free imaging of membrane potential using membrane electromotility- Biophysical Journal 103: (July) 11-18, doi: 10.1016/j.bpj.2012.05.020.

Ohshima H., S. Ohki- 1985-Donnan potential and surface potential of a charged membrane- Biophysical Journal 47: (May) 673-678.

Oldenhof H., M. Gojowsky, S. Wang, S. Henke, C. Yu, K. Rohn, W.F. Wolkers, H. Sieme- 2013-Osmotic stress and membrane phase changes during freezing of stallion sperm: Mode of action of cryoprotective agents- Biology of Reproduction 88: (#3, article 68) 1-11, doi: 10.1095/biolreprod.112.104661.

Oldham K.B- 2008-A Gouy-Chapman-Stern model of the double layer at a (metal)/(ionic liquid) interface- Journal of Electroanalytical Chemistry 613: 131-138, doi:10.1016/j.jelechem.2007.10.017.

Oliynyk V., U. Kaatz, T. Heimburg- 2007-Defect formation of lytic peptides in lipid membranes and their influence on the thermodynamic properties of the pore environment- Biochemica et Biophysica Acta 1768: 236-245, doi: 10.1016/j.bbamem.2006.10.007.

O'Neill M.J- 1966-Measurement of specific heat functions by differential scanning calorimetry- Analytical Chemistry 38: (#10, Sept.) 1331-1336.

Orth M., S. Bellosta- 2012-Cholesterol: Its regulation and role in central nervous system disorders- Cholesterol 292598, 19 pgs, doi: 10.1155/2012/292598.

Osterhout W.J.V- 1934-Nature of the action current in Nitella: I. General considerations - The Journal of General Physiology 18: (#2) 215–227, https://doi.org/10.1085/jgp.18.2.215.

Paajanen V., M. Vornanen- 2004-Regulation of action potential duration under acute heat stress by $I_{K,ATP}$ and I_{K1} in fish cardiac myocytes- American Journal Physiology - Regulatory, Integrative and Comparative Physiology 286: R405-R415, doi: 10.1152/ajpregu.00500.2003.

Paci M., L. Sartiani, M. Del Lungo, M. Jaconi, A. Mugelli, E. Cerbai, S. Severi- 2012-Mathematical modelling of the action potential of human embryonic stem cell derived cardiomyocytes- BioMedical Engineering Online 11: 61, 22 pgs., http://www.biomedical-engineering-online.com/content/11/1/61.

Páez P.L., M.C. Becerra, I. Albesa- 2013-Impact of ciprofloxacin and chloramphenicol on the lipid bilayer of *Staphylococcus aureus*: Changes in membrane potential- BioMed Research International article 276524, 5 pgs., doi: 10.1155/2013/276524.

Pagano R.E., R.J. Cherry, D. Chapman- 1973-Phase transitions and heterogeneity in lipid bilayers- Science 181: (#4099, 8/10) 557-559, doi: 10.1126/science.181.4099.557.

Paiva T.O., A.E.P. Bastos, J.T. Marqués, A.S. Viana, P.A. Lima, R.F.M. de Almeida- 2016-*m*-Cresol affects the lipid bilayer in membrane models and living neurons- The Royal Society of Chemistry Advances 6: 105699-105712, doi: 10.1039/c6ra20337j.

Pakhomova O.N., B. Gregory, I. Semenov, A.G. Pakhomov- 2014-Calcium-mediated pore expansion and cell death following nanoelectroporation- Biochemica et Biophysica Acta 1838: (#10) 2547-2554, doi: 10.1016/j.bbamem.2014.06.015.

Palti Y., W.J. Adelman jr.- 1969-Measurement of axonal membrane conductances and capacity by means of a varying potential control voltage clamp- Journal of Membrane Biology 1: 431-458.

Pauling L- 1957-College Chemistry. An Introductory Textbook of General Chemistry. 2nd ed. 685 pgs. W.H. Freeman and Company, San Francisco, U.S.A.

Peters J., J. Marion, F.J. Becher, M. Trapp, T. Gutberlet, D.J. Bicout, T. Heimburg- 2017-Thermodynamics of lipid multilamellar vesicles in presence of sterols at high hydrostatic pressure- Scientific Reports 7: 15339, 15 pgs., doi: 10.1038/s41598-017-15582-4.

Peyrard M- 2020-How is information transmitted in a nerve?- Journal of Biological Physics 46: 327-341, https://doi.org/10.1007/s10867-020-09557-2.

Pézolet M., D. Georgescauld- 1985-Raman spectroscopy of nerve fibers. A study of membrane lipids under steady state conditions- Biophysical Journal 47: (March) 367-372.

Pickard B.G- 1973-Action potentials in higher plants- The Botanical Review 39: (#2, April-June) 172-201.

Platkiewicz J., R. Brette- 2010-A threshold equation for action potential initiation- PLoS Computational Biology 6: (#7) e1000850, 16 pgs., doi: 10.1371/journal.pcbi.1000850.

Platt J.R- 1964-Strong inference- Science 146: (#3642, 10/16) 347-353, doi: 10.1126/science.146.3642.347.

Popper K- 2005-The logic of scientific discovery- 513 pgs., Routledge Classics, London, U.K.

Posada I.M.D., J.V. Busto, F.M. Goñi, A. Alonso- 2014-Membrane binding and insertion of the predicted transmembrane domain of human scramblase 1- Biochemica et Biophysica Acta 1838: 388-397, http://dx.doi.org/10.1016/j.bbamem.2013.09.018.

Post R.F- 1976-Nuclear fusion- Annual Review of Energy 1: 213-255.

Powell C.L., A.M. Brown- 2021-A classic experiment revisited: membrane permeability changes during the action potential- Advances in Physiology Education 45: 178-181, doi: 10.1152/advan.00188.2020.

Prenner E.J., R.N.A.H. Lewis, L.H. Kondejewski, R.S. Hodges, R.N. McElhaney- 1999-Differential scanning calorimetric study of the effect of the antimicrobial peptide gramicidin S on the thermotropic phase behavior of phosphatidylcholine, phosphatidylethalonamine and phosphatidylglycerol lipid bilayer membranes- Biochemica et Biophysica Acta 1417: 211-223.

Privalov G.P., P.L. Privalov- 2000-Problems and prospects of microcalorimetry of biological macromolecules- Methods in Enzymology 323: 31-62.

Rajendran M., B. Claywell, E.P. Haynes, U. Scales, C.K. Henning, M. Tantama- 2018-Imaging pH dynamics simultaneously in two cellular compartments using a ratiometric pH-sensitive mutant of mCherry- ACS Omega 3: 9476-9486, doi: 10.1021/acsomega.8b00655.
Rasband M.N., E. Peles-2016-The Nodes of Ranvier: Molecular assembly and maintenance- Cold Spring Harbor Perspectives in Biology 8: a020495, 16 pgs, doi: 10.1101/cshperspect.a020495.

Raudino A., M.G. Sarpietro, M. Pannuzzo- 2011-The thermodynamics of simple biomembrane mimetic systems- Journal of Pharmacy and Bioallied Sciences 3: (#1) 15-38, doi: 10.10.4103/0975-7406.76462.

Reece W.O- 2015-Body temperature and its regulation. Chapter 14, pgs. 149-154. In: Dukes' Physiology of Domestic Animals, 13th ed. W.O. Reece (Editor), H.H. Erickson (Associate Editor), J.P. Goff (Associate Editor), E.E. Uemura (Associate Editor). John Wiley & Sons, Inc. U.S.A.

Ritchie J.M- 1973-Energetic aspects of nerve conduction: The relationships between heat production, electrical activity and metabolism- Progress in Biophysics and Molecular Biology 26: 147-187.

Ritchie J.M., R.W. Straub- 1975-The movement of potassium ions during electrical activity, and the kinetics of the recovery process, in the non-myelinated fibres of the garfish olfactory nerve- Journal of Physiology 249: 327-348.

Ritchie J.M., R.W. Straub- 1956-The effect of cooling on the size and the action potential of mammalian non-medullated fibres- Journal of Physiology 134: 712-717.

Ros U., M.A. Edwards, R.F. Epand, M.E. Lanio, S. Schreier, C.M. Yip, C. Alvarez, R.M Epand- 2013-The sticholysin family of pore-forming toxins induces the mixing of lipids in membrane domains- Biochemica et Biophysica Acta 1828: 2757-2762, http://dx.doi.org/10.1016/j.bbamem.2013.08.001.

Roux B- 2017-Ion channels and ion selectivity- Essays in Biochemistry 61: (#2, 5/9) 201-209, doi: 10.104/EBC20160074.

Roy R., A.B. Das, D. Ghosh- 1997-Regulation of membrane lipid bilayer structure during seasonal variation: a study of the brain membranes of *Clarias batrachus*- Biochimica et Biophysica Acta 1323: 65-74.

Royer C.A- 2002-Revisiting volume changes in pressure-induced protein unfolding- Biochimica et Biophysica Acta 1595: 201-209.

Rudolph A.S., H.H. Crowe- 1986-A calorimetric and infrared spectroscopic study of the stabilizing solute proline- Biophysical Journal 50: (Sept.) 423-430.

Sabah N.H., K.N. Leibovic- 1972-The effect of membrane parameters on the properties of the nerve impulse- Biophysical Journal 12: 1132-1144.

Sahl S.J., M. Leutenegger, M. Hilbert, S.W. Hell, C. Eggeling- 2010-Fast molecular tracking maps nanoscale dynamics of plasma membrane lipids- Proceedings of the National Academy of Sciences U.S.A. 107: (#15) 6829-6834, www.pnas.org/cgi/doi/10.1073/pnas.0912894107.

Sanford F- 1899-The scientific method and its limitations- Address at the eighth annual commencement, Leland Stanford Junior University, May 24, pgs 3-22. Stanford University Press. U.S.A.

Sangrey T.D., W.O. Friesen, W.B. Levy- 2004-Analysis of the optimal channel density of the squid giant axon using a reparameterized Hodgkin-Huxley model- Journal of Neurophysiology 91: 2541-2550, doi: 10.1152/jn.00646.2003.

Sankaran J., T. Wohland- 2020-Fluorescence strategies for mapping cell membrane dynamics and structures- APL Bioengineering 4: 020901, 15 pgs., doi: 10.1063/1.5143945.

Savoie R., M. Pigeon-Gosselin, P. Pézolet, D. Georgescauld- 1986-Effect of the action potential on the Raman spectrum of the pike olfactory nerve- Biochimica et Biophysica Acta 854: 329-333.

Schneider M.F- 2021-Living systems approached from physical principles- Progress in Biophysics and Molecular Biology 162: 2-25, https://doi.org/10.1016/j.pbiomolbio.2020.10.001.

Schneider M.F- 2020-This is not about the molecules. On the violation of momentum conservation in biology- arXiv: 2004.10207[biophysics.bio-ph] and 204.10307v1. http://doi.org/10.1017/CBO9781107415324.004.

Schneider M.F., D. Marsh, W. Jahn, B. Kloesgen, T. Heimburg- 1999-Network formation of lipid membranes: Triggering structural transitions by chain melting- Proceedings of the National Academy of Sciences U.S.A. 96: (#25) 14312-14317.

Schrader W., H. Ebel, P. Graditz, E. Hanke, T. Heimburg, M. Hoeckel, M. Kahle, F. Wente, U. Kaatze- 2002-Compressibility of lipid mixtures studied by calorimetry and ultrasonic velocity measurements- Journal of Physical Chemistry B 106: 6581-6586.

Sebastián M, A.F. Smith, J.M. González, H.F. Fredricks, B. Van Mooy, M. Koblížek, J. Brandsma, G. Koster, M. Mestre, B. Mostajir, P. Pitta, A.D. Postle, P. Sánchez, J.M. Gasol, D.J. Scanlan, Y. Chen- 2016-Lipid remodelling is a widespread strategy in marine heterotrophic bacteria upon phosphorus

deficiency- The International Society for Microbial Ecology Journal 10: 968-978, doi: 10.1038/ismej.2015.172.

Sengupta P., J. Lippincott-Schwartz- 2020-Revisiting membrane microdomains and phase separation: A viral perspective- Viruses 12: 13 pgs, doi: 10.3390/v12070745.

Sezgin E., I. Levental, M. Grzybek, G. Schwarzmann, V. Mueller, A. Honigmann, V.N. Belov, C. Eggeling, Ü. Coskun, K. Simons, P. Schwille- 2012-Partitioning, diffusion, and ligand binding of raft lipid analogs in model and cellular plasma membranes- Biochimica et Biophysica Acta 1818: 1777-1784.

Shapiro M.G., K. Homma, S. Villarreal C-P. Richter, F. Bezanilla- 2012-Infrared light excited cells by changing their electrical capacitance- Nature Communications 3: 736, 10 pgs, doi: 10.1038/ncomms1742.

Shepherd G.M- 1988-The action potential- Chapter 6, pgs. 101-121, in Neurobiology, 2nd edition. Oxford University Press, New York, New York, U.S.A.

Shimmen T- 1997-Studies on mechano-perception in Characean cells: Pharmacological analysis- Plant Cell and Physiology 38: (#2) 139-148.

Shnyrov V.L., J.M. Sanchez-Ruiz, B.N. Boiko, G.G. Zhadan, E.A. Permyakov- 1997-Applications of scanning microcalorimetry in biophysics and biochemistry- Thermochimica Acta 302: 165-180.

Shrager P., S.Y. Chiu, J.M. Ritchie, D. Zecevic, L.B. Cohen- 1987-Optical recording of action potential propagation in demyelinated frog nerve- Biophysical Journal 51: (Feb.) 351-355.

Shrivastava S- 2021-Shock and detonation waves at an interface and the collison of action potentials- Progress in Biophysics and Molecular Biology 162: 111-121, https://doi.org/10.1016/j.pbiomolbio.2020.12.002.

Shrivastava S., H.J. Lee, J-X. Cheng- 2020-A thermodynamic interpretation of the stimulated Raman spectroscopic signature to action potential in single neurons- bioRxiv preprint, 12 pgs., posted on 4/23/2020, https://doi.org/10.1101/2020.04.20.052332.

Shrivastava S., R.O. Cleveland, M.F. Schneider- 2018a-On measuring the acoustic state changes in lipid membranes using fluorescent probes- Soft Matter (Nov.) doi: 10.1039/C8SM01635F.

Shrivastava S., K.H. Kang, M.F. Schneider- 2018b-Collision and annihilation of nonlinear sound waves and action potentials in interfaces- Journal of the Royal Society Interface 15: 20170803, 6 pgs., http://dx.doi.org/10.1098/rsif.2017.0803.

Shrivastava S., K.H. Kang, M.F. Schneider- 2014a-Solitary shock waves and adiabatic phase transition in lipid interfaces and nerves- arXiv posted preprint (21 pgs.) http://arxiv.org/abs/1411.2454.

Shrivastava S., M.F. Schneider- 2014b-Evidence for two-dimensional solitary sound waves in a lipid controlled interface and its implications for biological signalling- Journal of the Royal Society Interface 11: 20140098, 8 pgs., doi: 10.1098/rsif.2014.0098.

Shrivastava S., M.F. Schneider- 2013-Opto-mechanical coupling in interfaces under static and propagative conditions and its biological implications- PLoS One 8: (#7) e67524, (7 pgs.) doi: 10.1371/journal.pone.0067524.

Sierra-Valdez F.J., J.C. Ruiz-Suárez, I. Delint-Ramirez- 2016-Pentobarbital modifies the lipid raft-protein interaction: A first clue about the anesthesia mechanism on NMDA and $GABA_A$ receptors- Biochimica et Biophysica Acta 1858: 2603-2610, http://dx.doi.org/10.1016/j.bbamem.2016.07.011.

Simons K., D. Toomre- 2000-Lipid rafts and signal transduction- Nature Reviews Molecular Cell Biology 1: (Oct.) 31-41.

Sivils A., F. Yang, J.Q. Wang, X-P. Chu- 2022-Acid-sensing ion channel 2: Function and modulation- Membranes 12: 113, 21 pgs., https://doi.org/10.3390/membranes 12020113.

Sonnleitner A., L.M. Mannuzzu, S. Terakawa, E.Y. Isacoff- 2002-Structural rearrangements in single ion channels detected optically in living cells- Proceedings of the National Academy of Sciences U.S.A. 99: (#20, 10/1) 12759-12764, www.pnas.org/cgi/doi/10.1073/pnas.192261499.

Soto E., A. Ortega-Ramírez, R. Vega- 2018-Protons as messengers of intercellular communication in the nervous system- Frontiers in Cellular Neuroscience 12: 342, 16 pgs., doi: 10.3389/fncel.2018.00342.

Spyropoulos C.S- 1964-The role of temperature in the process of excitation of biological membranes- Il Nuovo Cimento 34: (#6, 12/16) 1837-1839.

Spyropoulos C.S- 1957-The effects of hydrostatic pressure upon the normal and narcotized nerve fiber- Journal of General Physiology 40: (#6) 849-857.

Srivastava R.C., S. Upadhyay, R. Sahney, R.L. Gupta- 1998-Investigations into the model of self sustained potential oscillations in lipid bilayers based on repetitive phase transitions- Journal of Membrane Science 143: 75-80.

Staves M.P., R. Wayne- 1993-The touch-induced action potential in *Chara*: Inquiry into the ionic basis and the mechanoreceptor- Austalian Journal of Plant Physiology 20: 471-488.

Steppich D., J. Griesbauer, T. Frommelt, W. Appelt, A. Wixforth, M.F. Schneider- 2010-Thermomechanic-electrical coupling in phosopholipid monolayers near the critical point- Physical Review E, 15 pgs., doi: 10.1103/PhysRevE.81.061123.

Street S- 2020-Upper limit on the thermodynamic information content of an action potential- Frontiers in Computational Neuroscience 14: 37, 7 pgs. doi: 10.3389/fncom.2020.00037.

Su L., J. Mosquera, M.F.J. Mabesoone, S.M.C. Schoenmakers, C. Muller, M.E.J. Vleugels, S. Dhiman, S. Wijker, A.R.A. Palmans, E.W. Meijer- 2022-Dilution-induced gel-sol-gel transitions by competitive supramolecular pathways in water- Science 377: (#6602, 7/8) 213-218, doi: 10.1126/science.abn3438.

Takashima S- 1979-Admittance change in squid axon during action potentials- Biophysical Journal 26: (April) 133-142.

Tamagawa H., K. Ikeda- 2018-Another interpretation of the Goldman-Hodgkin-Katz equation based on Ling's adsorption theory- European Biophysical Journal 47: 869-879, https://doi.org/10.1007/s00249-018-1332-0.

Tasaki I- 1982-Physiology and Electrochemistry of Nerve Fibers. 348 pgs. Academic Press. New York, New York, U.S.A.

Tasaki I., A. Watanabe, I. Singer- 1966-Excitability of squid giant axons in the absence of univalent cations in the external medium- Proceedings of the National Academy of Sciences U.S.A. 56: 1116-1122.

Tasaki I., I. Singer, A. Watanabe- 1965-Excitation of internally perfused squid giant axons in sodium-free media- Proceedings of the National Academy of Sciences U.S.A. 54: 763-769.

Tasaki I., A. Watanabe, T. Takenaka- 1962-Resting and action potential of intracellularly prefused squid giant axon- Proceedings of the National Academy of Sciences U.S.A. 48: 1177-1184.

Terakawa S- 1985-Potential-dependent variations in the intracellular pressure in the intracellularly perfused squid giant axon- Journal of Physiology 369: 229-248.

Theparambil S.M., P.S. Hosford, I. Ruminot, O. Kopach, J.R. Reynolds, P.Y. Sandoval, D.A. Rusakov, L.F. Barros, A.V. Gourine- 2020-Astrocytes regulate brain extracellular pH via a neuronal activity-dependent bicarbonate shuttle- Nature Communications 11: 5073, 15 pgs., doi: 10.1038/s41467-020-18756-3.

Thiel G., U. Homann, C. Plieth- 1997-Ion channel activity during the action potential in *Chara*: New insights with new techniques- Journal of Experimental Botany 48: (#s) 609-622, https://doi.org/10.1093/jxb/48.Special_Issue.609.

Tokarska-Schlattner M., R.F. Epand, F. Meiler, G. Zandomeneghi, D. Neumann, H.R. Widmer, B.H Meier, R.M. Epand, V. Saks, T. Wallimann, U. Schlattner- 2012-Phosphocreatine interacts with phospholipids, affects membrane properties and exerts membrane-protective effects- PLoS ONE 7: (#8) e43178, 11 pgs., doi: 10.1371/journal.pone.0043178.

Tolkien J.R.R-1973-The Lord of the Rings. Part one: The Fellowship of the Ring. 527 pgs. Ballantine Books. New York, New York, U.S.A.

Tomczak M.M., D.K. Hincha, S.D. Estrada, W.F. Wolkers, L.M. Crowe, R.E. Feeney, F. Tablin, J.H. Crowe- 2002-A mechanism for stabilization of membranes at low temperatures by an antifreeze protein- Biophysical Journal 82: (Feb.) 874-881.

Touska F., B. Turnquist, V. Vlachova, P.W Reeh, A. Leffler, K. Zimmermann- 2018-Heat-resistant action potentials require TTX-resistant sodium channels $Na_v1.8$ and $Na_v1.9$- Journal of General Physiology 150: (#8) 1125-1144, https://doi.org/10.1085/jgp.201711786.

Toyoda H., M. Saito, H. Sato, Y. Dempo, A. Ohashi, T. Hirai, Y. Maeda, T. Kaneko, Y. Kang- 2008-cGMP activates a pH-sensitive leak K^+ current in the presumed cholinergic neuron of basal forebrain- Journal of Neurophysiology 99: 2126-2133, doi: 10.1152/jn.01051.2007.

Tsai C-C., H-H. Hung, C-P. Liu, Y-T. Chen, C-Y. Pan- 2012-Changes in plasma membrane surface potential of PC12 cells as measured by Kelvin probe force microscopy- PLoS One 7: (#4) e33849, 7 pgs., doi: 10.1371/journal.pone.0033849.

Tsvetkova N.M., N.J. Walker, J.H. Crowe, C.L. Field, Y. Shi, F. Tablin- 2000-Lipid phase separation correlates with activation in platelets during chilling- Molecular Membrane Biology 17: (#4) 209-218, doi: 10.1080/09687680010013966.

Ueda T., M. Muratsugu, I. Inoue, Y. Kobatake- 1974-Structural changes of excitable membrane formed on the surface of protoplasmic drops isolated from *Nitella*- Journal of Membrane Biology 18: 177-186.

van Bilsen D.G.J.L., F.A. Hoekstra, L.M. Crowe, J.H. Crowe- 1994-Altered phase behavior in membranes of aging dry pollen may cause imbibitional leakage- Plant Physiology 104: 1193-1199.

van Osdol W.W., O.L. Mayorga, E. Freire- 1991-Multifrequency calorimetry of the folding/unfolding transition in cytochrome c- Biophysical Journal 59: (Jan.) 48-54.

Varela M., A. Roy, J. Lee- 2021-A survey of pathways for mechano-electric coupling in the atria- Progress in Biophysics and Molecular Biology 159: 136-145, https://doi.org/10.1016/j.pbiomolbio.2020.09.011.

Vargas E.V., A. Ludu, R. Hustert, P. Gumrich, A.D. Jackson, T. Heimburg- 2011-Periodic solutions and refractory periods in the soliton theory for nerves and the locust femoral nerve- Biophysical Chemistry 153: 159-167, doi: 10.1016/j.bpc.2010.11.001.

Vayghan H.S., S. Tavalaei, A. Grillon, L. Meyer, G. Ballabani, G. Glauser, P. Longoni- 2020-Growth temperature influence on lipids and photosynthesis in *Lepidium sativum*- Frontiers in Plant Science 11: 745, 13 pgs., doi: 10.3389/fpls.2020.00745.

Verkerk A.O., M.W. Veldkamp, A.C.G. van Ginneken, L.N. Bouman- 1996-Biphasic response of action potential duration to metabolic inhibition in rabbit and human ventricular myocytes: Role of transient outward current and ATP-regulated potassium current- Journal of Molecular and Cellular Cardiology 28: 2443-2456.

von Sachs J- 1906-History of Botany (1530-1860). Translated by H.E.F. Garnsey, and revised by I.B. Balfour. Second Impression. 568 pgs. Clarendon Press. Oxford University, U.K.

Wagensberg J- 2014-On the existence and uniqueness of the scientific method- Biological Theory 9: 331-346, doi: 10.1007/s13752-014-0166-y.

Wang L- 2012-Measurements and implications of the membrane dipole potential- Annual Review of Biochemistry 81: 615-635, doi: 10.1146/annurev-biochem-070110-123033.

Wang T., A. Gonzalez-Perez, R. Budvytyte, A.D. Jackson, T. Heimburg- 2017-Reply to "Comment on 'Penetration of action potentials during collision in the median and lateral giant axons of invertebrates'"- Physical Review X 7: 028002, 3 pgs, doi: 10/1103/PhysRevX.7.028002.

Wang T., T. Mužić, A.D. Jackson, T. Heimburg- 2018-The free energy of biomembrane and nerve excitation and the role of anesthetics- Biochimica et Biophysica Acta - Biomembranes 1860: 2145-2153, doi: 10.1016/j.bbamem.2018.04.003.

Watanabe A., I. Tasaki, L. Lerman- 1967-Bi-ionic action potentials in squid giant axons internally perfused with sodium salts- Proceedings of the National Academy of Sciences U.S.A. 58: 2246-2252.

Wayne R- 1994-The excitability of plant cells: With a special emphasis on Characean internodal cells- Botanical Review 60: (#3, July-Sept.) 265-367, http://www.jstor.org/stable/4354233.

Webber M.J- 2022-Less is more when forming gels by dilution- Science 377: (#6602, 7/8) 153-154, doi: 10.1126/science.abo7656.

Welti R., D.A. Rintoul, F. Goodsaid-Zalduondo, S. Felder, D.F. Silbert- 1981-Gel phase phospholipid in the plasma membrane of sterol-depleted mouse LM cells- The Journal of Biological Chemistry 256: (#14) 7528-7535.

Westaway F.W- 1919-Scientific Method: Its Philosophy and its Practice. 426 pgs. 2nd edition. Blackie and Son Limited. London, U.K.

Wieser V., L.L.E. Mears, RD. Barker, H-W. Cheng, M. Valtiner- 2021-Hydration forces dominate surface charge dependent lipid bilayer interactions under physiological conditions- The Journal of Physical Chemistry Letters 12: 9248-9252, https://doi.org/10.1021/acs.jpclett.1c02572.

Winter R., W. Dzwolak- 2005-Exploring the temperature-pressure configurational landscape of biomolecules: from lipid membranes to proteins- Philosophical Transactions of the Royal Society A 363: (#1827) 537-563, doi: 10.1098/rsta.2004.1507.

Wunderlich B., C. Leirer, A-L. Idzko, U.F. Keyser, A. Wixforth, V.M. Myles, T. Heimburg, M.F. Schneider- 2009-Phase-state dependent current fluctuations in pure lipid membranes- Biophysical Journal 96: (June) 4592-4597, doi: 10.1016/j.bpj.2009.02.053.

Xu X., A. Denic, A.E. Warrington, A.J. Bieber, M. Rodriguez- 2013-Therapeutics to promote CNS repair: A natural human neuron-binding IgM regulates membrane-raft dynamics and improves motility in a mouse model with multiple sclerosis- Journal of Clinical Immunology 33: (suppl. 1) 50-56, doi: 10.1007/s10875-012-9795-8.

Yagisawa K., M. Naito, K-I. Gondaira, T. Kambara- 1993-A model for self-sustained potential oscillation of lipid bilayer membranes induced by the gel-liquid crystal phase transition- Biophysical Journal 64: (May) 1461-1475.

Yeung T., M. Terebiznik, L. Yu, J. Silvius, W.M. Abidi, M. Philips, T. Levine, A. Kapus, S. Grinstein- 2006-Receptor activation alters inner surface potential during phagocytosis- Science 313: (#5785, 7/21) 347-351, doi: 10.1126/science.1129551.

Yi G-S., J. Wang, H-Y. Li, X-L. Wei, B. Deng- 2016-Metabolic energy of action potentials modulated by spike frequency adaptation- Frontiers in Neuroscience 10: (Nov.) 534, 19 pgs, doi: 10.3389/fnins.2016.00534.

Zecchi K.A., T. Heimburg- 2021-Non-linear conductance, rectification, and mechanosensitive channel formation of lipid membranes- Frontiers in Cell and Developmental Biology 8: (Jan.) 592520, 18 pgs., doi: 10.3389/fcell.2020.592520.

Zecchi K.A., L.D. Mosgaard, T. Heimburg- 2017-Mechano-capacitative properties of polarized membranes and the application to conductance measurements of lipid membrane patches- Journal of Physics, IOP Conf. Series: 780: 012001, 9 pgs, doi: 10.1088/1742-6596/780/1/012001.

Zhou X., L. Sumrow, K. Tashiro, L. Sutherland, D. Liu, T. Qin, M. Kato, G. Liszczak, S.L. McKnight- 2022-Mutations linked to neurological disease enhance self-association of low-complexity protein sequences- Science 377: (#6601, 7/1) 46, https://doi.org/10.1126/science.abn5582.

Zuckermann M.J., T. Heimburg- 2001-Insertion and pore formation driven by adsorption by proteins onto lipid bilayer membrane-water interfaces- Biophysical Journal 81: (Nov.) 2458-2472.