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Macroscopic Self-Organized Electrochemical Patterns in Excitable Tissue and Irreversible Thermodynamics

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Abstract

In this paper we make the assertion that the key to understand the emergent properties of excitable tissue (brain and heart) lies in the application of irreversible thermodynamics. We support this assertion by pointing out where symmetry break, phase transitions both in structure of membranes as well as in the dynamic of interactions between membranes occur in excitable tissue and how they create emergent low dimensional electrochemical patterns. These patterns are expressed as physiological or physiopathological concomitants of the organ or organism behavior. We propose that a set of beliefs about the nature of biological membranes and their interactions are hampering progress in the physiology of excitable tissue. We will argue that while there is no direct evidence to justify the belief that quantum mechanics has anything to do with macroscopic patterns expressed in excitable tissue, there is plenty of evidence in favor of irreversible thermodynamics. Some key predictions have been fulfilled long time ago and they have been ignored by the mainstream literature. Dissipative structures and phase transitions appear to be a better conceptual context to discuss biological self-organization. The central role of time as a global coupling agent is emphasized in the interpretation of the presented results.

Keywords

Brain Self-Organization, Non-Linear Thermodynamics, Membrane Phase Transitions, Memory and Learning

1. Introduction

In this paper we make the assertion that the key to understand the emergent properties

of excitable tissue (central nervous system and muscular tissue) lies in the application of irreversible thermodynamics.

In 1968 Ilia Prigogine and René Lefevre published the kinetic description of a chemical system with self-organization, later called the *Brusselator* [1]. Twenty years later, in 1988, Herman Haken [2] summarized the general methods and concepts involved in the field of self-organization in complex systems. He detailed the controversies as seen from the theoretical physicist point of view and stated that (at that point in time)… "there is still disagreement even about quite fundamental aspects. I do hope that my article will help to clarify the present status of the theoretical approaches to complex systems." …"We have two methodologies at hand, namely the macroscopic or phenomenological approach and the microscopic approach which attempts at deriving macroscopically observed phenomena from basic equations which in the case of chemistry must eventually be the physical laws established by quantum mechanics and electrodynamics." More than 20 years later the field appears to be at the same stage of knowledge. The main problem appears to be limitations of the mathematical methods. For example, a type of dissipative structure, that we believe is crucial for the understanding of all biology, is the electrohydrodynamic bridges (EHD) especially the water bridges studied by the group headed by Elmar C. Fuchs [3]. Water is the fundamental polar liquid in living organisms and the bridges are dynamic structures created by electrical and gravitational fields. We quote: "A number of common solvents can form such bridges as well as low conductivity solutions and colloidal suspensions. The macroscopic behavior is governed by electrohydrodynamics and provides a means of studying fluid flow phenomena without the presence of rigid walls. Prior to the onset of a liquid bridge several important phenomena can be observed including advancing meniscus height (electrowetting), bulk fluid circulation (the Sumoto effect), and the ejection of charged droplets (electrospray). The interaction between surface, polarization, and displacement forces can be directly examined by varying applied voltage and bridge length. The electric field, assisted by gravity, stabilizes the liquid bridge against Rayleigh-Plateau instabilities." Fuchs uses classical electrodynamics to describe theoretically his findings; however, he also states clearly the limitations of continuum methods: "…These theoretical approaches suffer certain limitations which should be considered when approaching experimental data. The Maxwell stress tensor treatment is insensitive to field heterogeneities as well as non-uniformities in the liquid bridge. A pure EHD approach provides steady state definitions of the electrogravitational number and its relationship to the bridge aspect ratio; however, the flow dynamics and important transient phenomena (e.g., bridge creation) are not predicted." Another important observation not predicted is the emission of non-Plank infrared radiation by the bridge water [4]. The lack of prediction of transients and the sudden changes observed in physicochemical systems are still the main theoretical problems in self-organization of complex systems. Neither classical nor quantum mechanics explain the transients.

The first twenty years in this field were characterized by and explosion of interest

and the close cooperation between applied mathematicians and experimenters in physical chemistry and biology, along with an ever expanding computer power. The basics were set: both experimental models and simulations showed that the same system could display oscillations and spatial patterns formation (waves in excitable media) depending on experimental context. The oscillatory regimen also was rich in displaying different behaviors, from an almost limit cycle to deterministic chaos. Popular models were (and still are) the glycolytic cycle in yeast, the behavior of the slime mold and the chemical system of Belouzov-Zabotinsky. The promise was that a revolution in physiology was around the corner. However, this journal published a review in 2014 [5] (Self organization and Coherency in Biology and Medicine, by Goushcha, A. *et al.*) in which the theory is still at the same point that it was at 1988 and the great advance in medicine and biology did not come. Why? Well, a possibility is that some strong held beliefs are just that, beliefs and not knowledge. For example, the belief that quantum mechanics will explain all macroscopic phenomena. If that is so, why it is that the Hofmeister series did not become obvious from quantum mechanics already in the last century? Still now the interfacial effects of ions on polylectrolites lack a full explanation. Much worse is the belief that wonders of the mind have anything to do with the highly abstract collapsing waves defined deterministically in Schrodinger´s equation. A deeper belief underlies the second one: that Mathematics is a Revelation of absolute Truth and Beauty. In other words, the implication that mathematical concepts are discoveries and not inventions. We hope that it is clear that neither the importance nor the usefulness of mathematics is challenged here, only the belief on its sacredness.

The origin of the popularity of the generalization of quantum mechanics concepts to mind is the famous book by Roger Penrose the Emperor's New Mind [6]. In the following book he shows the influence of Stuart Hameroff faith in the power of microtubules as the conveyors of high mental functions [7] [8]. The only shred of direct evidence about microtubules storing energy we think is a misinterpretation of experimental results [9]. The fact is that a single *wet* human microtubule did store energy and showed resonances. The authors, in our opinion, measured the properties of interfacial water instead of a particular microtubule effect. First, there is little doubt now about pure interfacial water storing electrical energy [10]-[12]. Second, this energy appears to be related to a macroscopic state of water that behaves optically as quasi-liquidcrystals do [13]-[15]. Apparently this energy can be dissipated as infrared radiation [4] [16] or mechanical flow [3] [17]. We will propose that this energy from dissociated water is an important part of blood flow in venules, the spread of action potentials in axons, the heart beat and spreading depression waves and other electrochemical patterns in the brain present in functional syndromes. Maybe they could also explain perception and memory.

We are no theorists but tinkers, and as such, believe that experiments (especially our own) should not only agree with theory, but when they disagree, experiments should have precedence. In this paper we will show experimental results and interpret then in the irreversible thermodynamical context.

2. In Excitable Tissue, the Symmetry Break Is at the Structural Level

One aspect of biological membranes that gives raises to little discussion is the significance of the asymmetry of the membranes. It begins at the lipid bilayer level and is extensive to the continuous gels that form the extracellular matrix and the cytoplasm.

The lipid bilayer has both zwitter-ionic (phosphatidylcholine andphosphatidyl-ethalonamine), and charged (phosphatidylserine) fatty acids asymmetric distributed in both leaflets. Simulation showed that this asymmetry alone contributes to the self-organized electric field of biological membranes [18]. The other component of biological membranes is the gel made of the polyelectrolites. At the external leaflet, we have glycolipis (gangliosides) depicted as fatty acids with protruding "trees" of the polar sialic acid. Also we have the intrinsic membrane proteins of receptors, channels and the Na/KATPase, all glycoproteins with the same branches of sugars protruding from the external leaflet. Continuum to that are posed the chains of polysugars called Glycosaminoglycans (GAGs). All of these polar polymers are polyanions polyelectrolites. As a consequence, we have several layers of ionizable negative charges at the external leaflet with different degrees of freedom to move. The frontier between external membrane gel and the gel of the extracellular matrix is rather obscure. At interfaces in brain and muscle, a large (~200 μm extension) polyelectrolyte in which the GAGs predominate, receives the name of basement membrane. Another name for the charged gel is glycocalix. Here we will focus on the fact that all of them are negatively charged polyelectrolites. The estimated electrical field of axonal membranes is of the order of 100,000 V/cm^2, a rather large self-organized field. Aharon Katchlasky estimated the electrical field of biological polyelectrolytes at 300,000 V/cm^2 [19]. The gap between the membranes can be something from 20 to 60 nm wide within the neuropil. These are the spatial dimensions influenced by the self-organized electrical fields. In a classical chemical synapse, acetylcholine (ACh) will be an ion. To think that ions behave in the extracellular space has they do in diluted solutions, is just plain wrong. A recent publication shows the glial network in retinas and in cortex with the depiction of the convoluted small space between membranes within the neuropil and the contiguous gel that covers the endfeet of glia at interfaces [20]. At all central grey matter interfaces, glial endfeet are covered by basement membranes and the extracellular gel fills the convoluted interval between cell membranes.

In his chapter Haken makes the good point that the concept of irreversibility is linked to some form of transport of either mass or energy [2], of which Fick's law is just a particular case. Nevertheless "reaction-diffusion" models are used in all biological simulations (see for example Zykov, 2008 [21]). The mathematical convenience of the application of Fick's law, is easy to understand; however, to describe what goes on with in the neuropil, it does look like the proverbial perfectly spherical cow of the anecdote. Nevertheless, researches still think about the space as a diluted solution [22]. By contrast, Aharon Katchalsky could visualize coherent flow at synapses due to the properties of polyelectrolites [19]. Macroscopic coherent flow is induced by the interaction between pure water and polyelectrolytes (nafion) [17]. The dissociated interfacial water

acquires macroscopic structure (see for example [23]). This structure has birefringence; indeed its optical behavior suggested the orderly packing of quasi-liquidcrystals [13] [14]. Nafion is a polyanion (sulfite anions) the biological counterpart is keratan sulfate, a GAG major component of basement membranes and extracellular matrix, the other polyanions at external leaflet are poliacids (hyaluronan, gangliosides).

The internal leaflet of biological membranes interacts with the cytoskeleton and a system of internal membranes that compartmentalize the space. The cytoskeleton is a number of fibrils (polymers of actin and other acidic proteins called intermediate filaments) and the famous microtubules. Finally, the cytoplasm has organelles as the mitochondria and the nucleus. The predominant inorganic anion is phosphate. The hallmark of glia is the Glial Fibrillary Acidic Protein. It makes the intermediate filaments of glia in retinas. Their shape suggests the ideal configuration for inducing a ring of dissociated water around them. It is tempting to speculate that this configuration could explain the confirmed light guide property of the radial glia in vertebrate retinas [24] [25]. The birefringence of the structured water shell could be explored in this way by retinas and guide light to the receptors.

In summary, biological membranes are asymmetric and this asymmetry builds an intense self-organized electrical field; therefore, electroneutrality does not exist close to membranes.

3. The Minimum Requirements for the Propagation of Two Dimensional Electrochemical Waves of Biological Significance

In **Figure 1** we show the circular spread of a solitary retinal spreading depression wave.

Note the small dots marked with an asterisk and the homogeneous spread of the wave. The black structure is called pecten and it is present in avascular retinas like the chicken. It is composed of pigmented epithelium and vessels and it is continuous with the sclera. At the front of the pecten lies the optical papilla, the place where the axons of the ganglion cells get together to form the optical nerve. It has the shape of a half moon and at the center is about 250 μm from the pecten [26] [27]. In this part of the retina only the radial glia and axons are present; no synaptic contacts, no neural cell bodies with their organelles can be found. Note that this drastic change in tissue structure does not affect the smoothness of the wave spread or the intensity of the optical signal. This observation was published first in 1966 [28]. It should also be noted that the pharmacological blockade of action potentials with TTX does not affect retinal excitation waves in any parameter measured (see for example [20] [29]); therefore, there is no contribution of the axons to spread velocity and intensity of the optical signal of the waves. What are left are the glial network and the associated polyelectrolyte of the basement membrane that covers the endfeet of glia and the polyelectrolyte between glial cells and axons. The only possible conclusion is that interacting membranes through the charged gel are the minimum requirements for two dimensional electrochemical waves in excitable tissue. The work of Manfred Wussling and collaborators with two dimensional calcium activity waves corroborates this interpretation [30] [31]. They used agarose gel and membrane fragments from endoplasmic reticulum and mitochondria from muscle

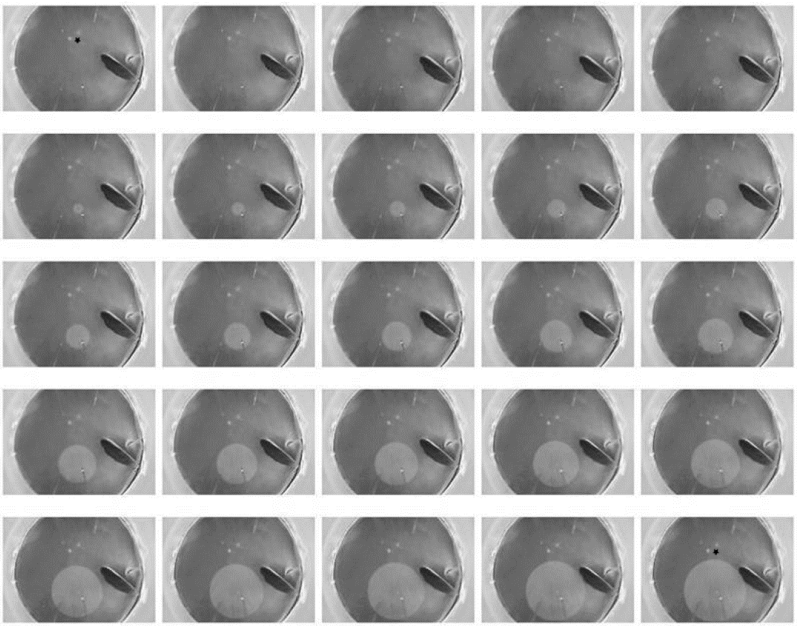


Figure 1. The sequence of frames show the temporal evolution of a solitary circular wave of retinal spreading depression. Despite the name it an excitation wave. The interval between each frame is of 5 seconds. The total time 125 seconds. The asterisks at the first and last frames show microlesions that always develop after a mechanical touch that elicits waves. The black structure is the pecten (7 mm length) and the eyecup diameter is 13.5 mm. Note that the circular pattern of the propagating wave just ignores the drastic change in the tissue histology at the optical papilla that it is continuous with pecten, in front of it. At this place, only the axons of ganglion cells and glia are present covered by the basement membrane (polyelectrolyte).

cells, creating the perfect reductionist model for electrochemical patterns relevant for physiology.

More corroborating evidence about the role of the external polyelectrolytes, comes from the experiments with protamine in axons, retinas and epithelium and the serendipitous results of protamine in a human transplanted heart [32]-[34]. In other words, protamine blocked spread of excitation in one (axon), two (retinas) and in three dimensions severely impaired the heart beat spread and force. To our delight, studies of effects of protamine on basement membranes showed that it changed the structure of the basement membrane without affecting gap junctions. Neither did it interfere with membrane potential of epithelium. In retinas it brought the collapse of excitability at nanomolar range within minutes of its application in the perfusion system. It appears that the effect of the cationic protein on excitability is due to the formation of rather large heteropolymers complexes with heparan sulfate, a GAG. Essays *in vitro* with plasma, showed that these heteropolymers were large, estimated at 100 nm in size [35]. In retinas protamine did not alter any parameter of the optical profiles of waves obtained with the system in the route of excitability collapse, only its size, the last wave before collapse looked like a miniature of the controls. This strongly suggests that the

heteropolymers were far from the lipid bilayer. Besides, during this time transparency of the tissue increased: in retinas, transparency and good physiological conditions go hand in hand. We interpreted these findings as a mechanical clamping of the basement membrane by the heteropolymers that hindered more and more areas hampering wave spread.

Two lessons can be learned from these observations:

a) The role of gap junctions is at best overrated in the spread of excitation in tissue; by contrast, the integrity of the basement membrane is underrated; this is a clear example of strong belief influencing a whole research field. For example the bold title "Gap junctions are required for the propagation of spreading depression" used by Mieke Nedergaard and collaborators [36], is an example of bold belief. Unhappily for them, Müller cells in the chicken retina haveno gap junctions (see [29]). Furthermore, they based their results on the fact that alcohols appear to block the wave spread. However, their experiments have a terrible mistake: they used phosphate buffer and this buffer kills retinas or any tissue under illumination, due to the fact it creates H_2O_2 and the free radicals are deadly [37]; to make matters worse, they used O_2 gas to bubble the perfusion solution. In short, they had fast dying retinas to begin with. We know from different series of experiments that propanol and octanol does not block wave propagation, octanol accelerates tissue metabolism and increases propagation velocity [38] [39]. Underlying the belief that gap junctions are important for propagation is the belief that they are needed in order to have a syncytium behavior (just look the definition at Wikipedia).

b) The only model of membrane excitation that requires the external leaflet of membranes, is the Ichigi Tasaki action potential model (see below), indeed his model requires phase transitions in the polyelectrolites that we will discuss in the next section. It is the only model that can explain all the results of protamine and spread of excitation. Indeed, after our experiments and reading the findings in axons and epithelium in the literature, we could predict the same effect in the heart and found the case report on a human *transplanted* heart, meaning that the effect had to be direct to the muscle. Correct prediction strongly corroborates scientific conjectures.

The idea that classical diffusion is at the heart of excitation spread in tissue simply does not agree with experiments. **Figure 2** shows how a microlesion (small circle about 50 μm) splits a circular wave like the one shown in the previous figure.

In that figure we called attention to the small lesions that signaled mechanical touches used to elicit waves. No matter how light the touch, a lesion will develop on the region. It develops slowly taking several minutes to be clearly seen. Occasionally, such lesions develop around the electrode tip inserted in the innerplexiform layer (or retinal neuropil). When this happens, the electrode is "blind" to the potential drop caused by one wave invading the region. When this happens, the electrode has to be moved away. That what happened in the experiment showed in **Figure 2**. The figure shows the result of subtracting two consecutive frames separated by one second interval. Note that subtraction result shows the front-wave active zone. In this case, it has 50 μm, what gives spread velocity of 3 mm/min the typical wave velocity of retinal experiments. Note how

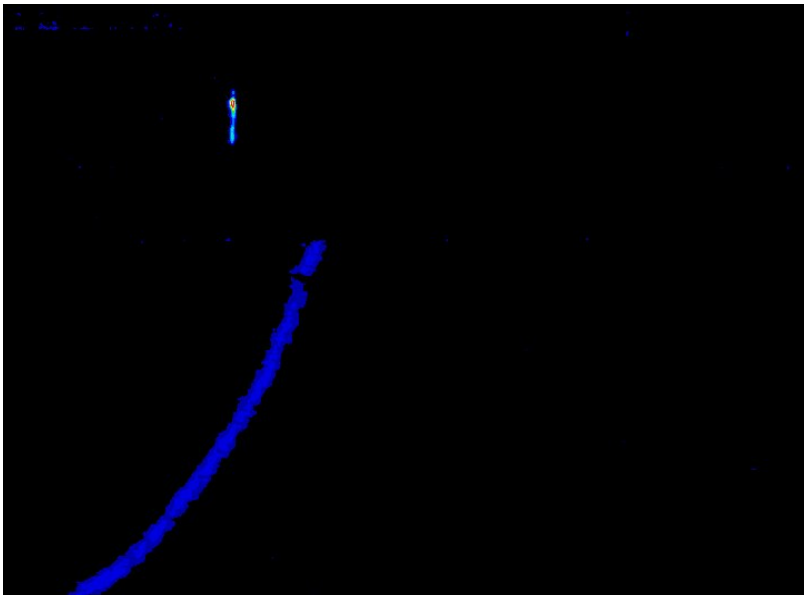


Figure 2. The frame shows the result of the subtraction of two consecutive frames of an evolving circular wave. The time interval between the frames was 1 second. The split on the wave-front was made when the wave reached a microlesion (about 50 μm) as shown in the previous figure. The result of the subtraction shows the active zone of the propagating wave. In this case it also has 50 μm. The result propagation velocity is 3 mm/min. Above is the result of the difference in the time series recorded simultaneously with the evolving wave (to be seen in **Figure 6**).

the split wave has different borders, the circular spread "forgotten" by the system. We interpret this finding in the following way: The touch irritates the tissue and the anionic gels undergo a slow folding around itself until volume collapse. Around this cylinder of collapsed tissue, there is another of free solution, the structured water that maintained the gel, reverts to liquid water and the inorganic ions will be distributed part to the solution part to the folded polymer. Calcium for example tends to insert into polymers and to form aggregates. By contrast, magnesium can only be immobilized in restricted spatial configuration (octahedral) [40]. The immobilization of the charges if it happens around an electrode tip, will make the electrode "blind" to events even as far as 50 μm away. We have more experimental evidence that gaps in the gel stops propagation no matter how thin the gap [41]. Also the circling wave experiments clearly show that the inner circle of tissue only rarely shows waves, the solution gap between inner circle and outer ring of tissue acting as a barrier to waves (see the arrangement of circling waves and more discussion in [29] [42]). Taken together the evidence appears to point to a crucial role for the structured dissociated water in the spread of excitation waves in tissue. Within seconds after the split, the smooth circular front-wave is restored. Indeed in the next second the two independent fronts collide and merge into one. This sequence of events just shows why a small infarted region does not impair the heart beat spread, and the integrating power of the irreversible thermodynamical context to explain events at one, two and three spatial dimensions. For example, a simple model of

self-oscillating system was created by combining the Belouzov-Zabotinsky reaction with a pH sensitive polyelectrolyte [43] [44]. The authors called the model a "simple heart".

4. The Irreversible Thermodynamics of Phase Transitions in Physiology

Two types of phase transitions are of interest in the physiology of excitable tissue: Lipid bilayers phase transitions and volume phase transitions of polyelectrolites. We will begin with the lipid bilayer phase transitions.

4.1. The Lipid Bilayer as a Model and as Part of the Biological Membranes

In short, biological membranes are highly complex structures, mainly composed of lipids, associated and integral proteins, and some polyelectrolites. Usually, functional aspects of these membranes are associated with the proteins, and the question how the fundamental properties of the lipid core of the membrane influence its general properties has not received too much interest in the scientific community (even now?). This approach became even more popular with all the success of molecular biology (*i.e.*) [45], the patch-clamp technique [46] and new optical technologies [47]. Nevertheless, it should be obvious, that the lipid matrix of a membrane significantly contribute to its functions just due to physical reasons. Trivial and well accepted is that the pure lipid core is a reasonable electrical isolation, being impermeable to ions and hydrophilic molecules, and that the electrical capacity of a membrane in some extent contributes to the electrophysiological properties of membranes, including action potential behavior.

However, having a deeper look at the contribution of the lipid core to membrane properties, the situation becomes a bit more complicated. In the early 70th and 80th of the last century a variety of scientists speculated for example that the plain lipid core might significantly contribute to the permeability properties of membranes by the existence of pore like ionic conductances in plain lipid phases [48]. There were also theories, asking, whether for example esterase induced pH changes might be mainly involved in the behavior of (cholinergic) synaptic transmission [49] [50]. Finally it was even questioned whether the Hodgkin-Huxley-model of action-potential behavior was correctly describing the situation [51].

This became even more important when it was shown that, plain lipid bilayers can undergo well defined phase transitions [52] [53] and that the conducting properties of plain lipid bilayers during such phase transitions are significantly changed. Two aspects were of interest here; first, plain lipid bilayers exhibited an increase of membrane conductance, later interpreted as pore like fluctuation, in the range of the phase transition temperature [54], and second, protein mediated membrane transport processes are significantly changed [52] in the range of the phase transition. Furthermore, membrane capacity increased in the frozen state of the membrane as expected.

Although the facts in itself were accepted by the scientific society, some further going interpretations especially concerning synaptic transmission, action potential onset and

propagation, and the structure of ionic channels [48] were not that well accepted, not to say, they were widely ignored. Additionally, when later looking at biological membranes and plain lipid bilayers under the aspects of non-linear thermodynamics [55] [56] it became obvious that even plain lipid bilayer might be seen as thermodynamically energized excitable media with consequences such as them being critically dependent on small external forces or exhibiting self-organization, oscillations and propagating waves and pulses, one theoretical approach also not that much tolerated at the early times.

We will not go that far here, as some scientists in early times did, to state that protein contribution in membrane properties is highly overestimated, but just want to point out that the physical state of the lipid core clearly affects all properties of proteins and thus their structure and function and hence membrane function, especially membrane transport, in general. Second, as any biological membrane is a highly complex structure as stated above, usually it is not homogenous, and locally occurring phase transitions, possibly induced by protein-lipid interactions or other small environmental forces [57], might occur even under physiological conditions, therefore giving rise to processes as stated above.

Historically seen, after some first discussions in the 80th of the last century, not very much happened in the lipid bilayer scene until more recently when some new groups entered the field and again demonstrated clearly the importance of the lipid core and the phase transition contribution to the behavior of biological membranes, especially to the electrical properties of membranes and membrane transport phenomena.

Detailed information has recently been published about the mechanisms of lipid ionic channels as being a consequence of fluctuations in area and compressibility close to lipid phase transitions [54] [58] [59]. The effects of a variety of substances such as *i.e.* anesthetics introducing further pore like events in membranes by changing the lipid core structure [60] has been described and its importance in the field of pharmacology and medicine became obvious. It has also been shown that changes in the bulk pH or of other bulk parameters might induce ionic conductance's in bilayers [49] [50] [61] or at least change their properties. As a consequence the discussion about the real nature of cholinergic synaptic transmission is on again [61]. Interestingly, it has been shown that the distinction between protein-mediate ion-channels and lipid ion channels is not possible under certain conditions [62], a finding of high importance when generalized according to the mechanistic nature of ion-channels.

The existence of the propagation of electric pulses in lipid monolayers has then induced again the question about action potential mechanisms, and thus alternative mechanisms to the Hodgkin-Huxley-model [63] like a soliton-model or a plain (possibly even reversible-what we think is wrong-) thermodynamical interpretation are under discussion again.

Finally it has even been shown that plain lipid membranes interact with small external forces, such as gravity [64], verifying to treat them as excitable media, as long as they are far from equilibrium for example by applied potentials or substance gradients

across them. This implies all the following consequences: plain lipid membranes might exhibit pattern-formation, self-organization, propagating waves and pulses, and oscillations. There properties might be changed by small external forces, the chemical composition of the boundary layers, changes in the structured water layers at the hydrophobic boundaries of the membranes, or changes in the electrical field across the membrane. Of course also proteins will change membrane properties without any contradiction to a non-linear thermodynamical approach to membrane function.

4.2. Volume Phase Transitions in Polyelectrolites and Excitable Tissue Physiology

We now turn to polyanions phase transitions. The pioneer in studying polyelectrolites in relation with action potentials in axons is Ichigi Tasaki [65]-[68]. He first found (1965) that if most of the cytoplasm is extruded and the axon tube filled with inorganic solutions, excitability could be maintained for several hours, showing that membrane and not the cytoplasm have the property of excitability. Furthermore, he found that the ions could be ordered in relation to favorability of excitation and that this sequence just followed the Hofmeister series of colloidal physical chemistry. Later on he studied the effects of ions on the volume phase transitions of polyanions. In his last paper (2008) [69] he summarized what he learned about action potentials… "***The propagating nerve impulse is a running wave of reversible structural change, representing a continuous displacement of the boundary between the site of swelling and the site of shrinkage of the cortical gel layer.***"

In the retinas several observations are suggestive of phase transitions. In the previous section we showed a slow developing microlesion, in here we will show that microlesions can appear suddenly, just like the popping of popcorns. In two occasions we were lucky that this happened around the electrode inserted in the neuropil. **Figure 3** and **Figure 4** show the potential and calcium activity associated with the events.

Both experiments were circling wave experiments and in both cases the electrode was repositioned in the same meridian about 1 mm apart from the original site. Careful inspection of the figures show that in both experiments quantitative and qualitative changes were seen before the explosive microlesions: the amplitude of the wave concomitants got smaller and in the calcium electrode experiment, the circling stopped altogether.

Also in **Figure 3** the potential drop (potentials drops are shown in the upward direction for historical reasons in electrophysiology) associated with the lesion happened when the tissue should be at the absolute refractory period (at 30˚C this period is 2.5 minutes). Now we interpret these observations as volume phase transitions of the external polyelectrolyte associated with glia membrane and the fall of activity of calcium as explained, in the most part, by the ion immobilization within the polyanions rather than calcium moving through channels into cells. Microlesions in retina are visible and they have different modes to reach final state: a slow (several minutes) and a fast (seconds) emergence, both interpreted as volume collapse of the external polyelectrolites and loss of the structured water. The release of structured water liberates energy.

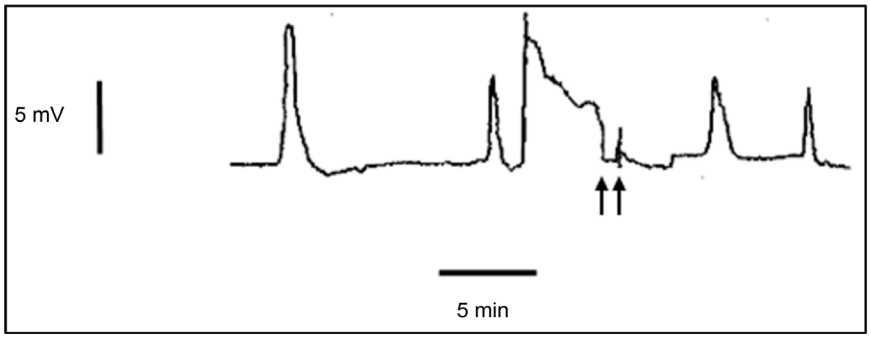


Figure 3. Continuous pen recorder trace of an experiment with a self-sustained retinal wave trapped in a ring of tissue. It is a raw trace of the extracellular potential drop (potential drops are shown upward) associated with the wave reaching the microelectrode tip inserted in the inner-plexiform layer of a retina (neuropil). Note the long interval between the first and second wave and the loss of amplitude in the second wave. Then suddenly a potential drop was recorded associated with a bright spot that popped up and signaled the birth of micro-lesion in the tissue around the electrode. The electrode was then withdrawn and inserted at the same meridian of the circular ring 1 mm away from the lesion. The circling wave reached this new position of the electrode and two successive waves are shown. Note that the rise of the potential wave of the lesion is similar to the potential drops of circulating waves, however the recovery is slower. We interpret the rise of the bright spot as a sudden collapse of the external gel.

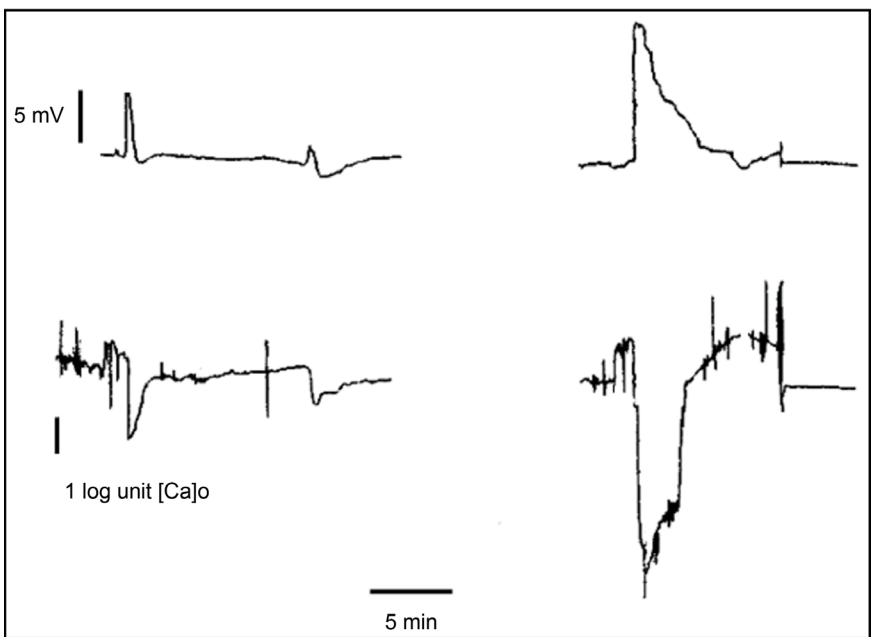


Figure 4. A circling wave experiment as in the previous figure. Inserted in the neuropil was a calcium sensitive micro-electrode. Associated with the waves the extracellular potential drop and calcium ion activity were measured simultaneously. On the left the record shows the two last waves of a circling wave experiment. Note that the last wave had low amplitude and a different kinetics from the previous patterns, the usual pattern recorded in retinal waves. The circling wave stopped a minutes later a sudden micro-lesion developed around the electrode tip. The electrophysiological potential drop and calcium activity are shown in right of the record. The fall in calcium activity and its duration are of larger amplitude and longer duration. The sudden collapse of the gel appears to sequester calcium.

Back in 1993 when preparing for experiments with voltage sensitive dyes, we discovered that the light scatter of retinal waves had two different components at the short (blue) and long (red) wavelengths. Using two cameras with optical filters in front of them, we took images of the transition from quiescence to the beginning of propagation. The cameras were high sensitivity cameras and their range included near infrared (700 nm). We also made images with an infrared camera (shown in [29]). Here we will interpret this data after the findings with macroscopic interfacial water and its storage and release of electrical energy. At the critical transition from quiescence to excitation, amplifications and slowdown of oscillation are predicted. The video of Elmar Fuchs [3] show these oscillations beautifully in water bridges. We showed them in the intrinsic optical signal or red scatter [29] [70]-[72].

At the time we also discovered that a red band signal was present, even when the illumination was UV and the signal extrinsic fluorescence at the green band. We attributed this intrinsic signal entirely to scatter of fluorescent light originated at the outer retina (receptors and pigment epithelium). Now, we think that most of this signal originates from the interfacial water and represents release of energy from the quasi liquid crystal state to bulk liquid. This energy release is also expressed in the potential drop that hallmarks the invasion of a region by a wave-front. In **Figure 5** we show one of this

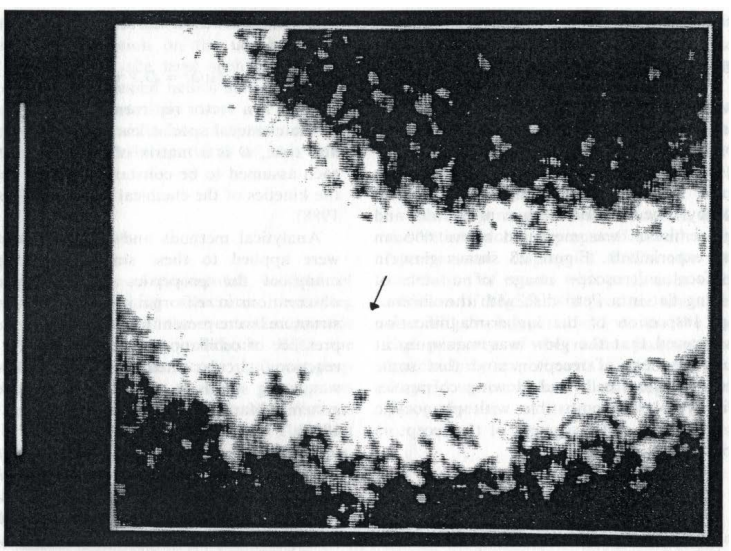


Figure 5. The frame shows a propagating circular retinal wave. Two sources of illumination were used in this experiment: an UV lamp (360 nm) and a xenon lamp with filtered output centered at 420 nm (20 nm filter width). The frame is the result of a background reference frame subtraction. The arrow show the propagation direction The bright continuous signal is the blue scatter of the wave. Note that the invasion of the quiescent tissue in the front is discontinuous and the fluctuations at the front of the wave are scatter of intrinsic red light (lesser brightness) and red plus the blue light (bright spots) at the points of potential drops. The recording camera was sensitive to near infrared. The border of the continuous blue signal is associated with the peak of the dV/dt extracellular signal. The white bar on the side is 1 mm long. Modified from [29].

type of experiments in which two sources of illumination were used: an UV lamp and a halogen lamp associated with an optical filter centered at 420 nm (20 nm bandwidth). The continuous optical signal is the blue band signal, in the front of it we have the active zone of the propagating wave or the fluctuations that only have long wavelength light.

The smooth green signal border shows the place of the propagation. Details of how the fluctuations behave in the latency interval between stimulus and propagating wave, the relation of calcium signal and the red scatter can be found in [29] [70]-[72]. The intrinsic optical signal of retinas shows that the transition from quiescence to excitation has all the hallmarks of a phase transition in the qualitative dynamics of the system. If the membrane Tasaki model is assumed to interpret the data, volume phase transitions in polyelectrolyte's are the key to understand triggering and propagation of excitation waves. The explanation for the split wave at **Figure 2** is that only one intact gel can spread volume phase transitions whereas bulk liquid water solutions hinder the propagation.

Another set of experimental results that can be reinterpreted following the findings of George Pollack [10] [17], Nicolai Bunkin [13] [14] and Elmer Fuchs [4] [12] [16], is the effect of glycerol on the optical signals of retinal waves: glycerol do not affect the rise of the potential drop, but in 50% of the experiments, it slowdown the recovery phase of the potential wave. In general amplitude of potential drop correlates positively with wave spread velocity. However, glycerol slowdown considerably the wave propagation velocity; irrespective of its lack of effect on the potential drop amplitude. It leaves the red scatter part of the wave signal intact but depresses strongly the brightness of the dominant blue/green component. The effects of glycerol are shown in [29] [72] [73].

Glycerol has a low partition coefficient for lipids and thus it is expected to remain close to the polar heads of the fatty acids, the place where structured dissociated water lies. Glycerol makes electrodynamics bridges and thus, can contribute to the ionized layer close to the heads, hence the little effect on the red scatter signal or the potential drop. On the other hand, glycerol depressed the green (488 nm) light scatter at unilamellar vesicles [74]. A possible interpretation of all these findings is that the propagation onset coincides with a phase transition at the lipid bilayer whereas the red signal shows structural change at the membrane external polyelectrolyte. The effect on propagation velocity could be due to the difference in viscosity between water and glycerol. Glycerol stretches the optical profile of waves slowing down recovery of the first optical component [72] and separating the two peaks of the profile (usually at 30˚C the peak interval is 2.5 min with glycerol it could be 7.5 min see [72]). The interpeak interval of retinal waves optical profiles shows the absolute refractory period of the system. For example, barium increases the absolute refractory period to 4 min at 30˚C [39] and also slowdown propagation velocity. However, barium depresses greatly the potential drop concomitant with waves. The barium effect of course is not related to effects on lipids, but is related to the Hofmeister effects of ions on polyelectrolites. It appears that inter-

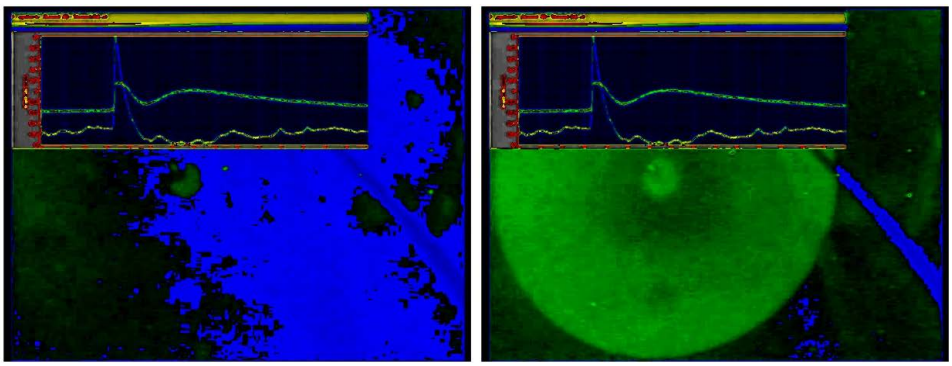
ference with either component of the phase transitions in membranes will affect the macroscopic concomitants associated with waves in different ways.

Last but not least, a piece of indirect evidence for the role of dissociated structured water and excitability comes from experiments in which liquid deuterium was used as solvent. The solvent deuterium was applied to retinas and to Belouzov-Zabotinsky (B-Z) reaction systems [75] [76]. Liquid deuterium has the surface tension and dielectric constant very similar to liquid water, however its viscosity is 25% higher than water and the self-dissociation coefficient 5 times smaller than water (pH at 25˚C is 7.41) [77]. The qualitative effect in both systems studied was remarkable: there was excitability collapse within the first hour of deuterium application. The effect of deuterium in B-Z was observed in bulk reactions, thin liquid layers (less than 2 mm thick) and silica gels experiments. All the experiments were short lived and the systems collapsed. In the gels, only a small part of the gel became active and the propagating waves died before reaching the border of the dish. In the liquid layer, rare standing patterns and giant solitary waves were observed. Seven retinas were exposed to the deuterium solution. Two were dead within the first hour of the experiment; the other five became instable and produced a series of "spontaneous" waves in the road toward excitability collapse. Total collapse happened also after one hour of deuterium exposition. Washing over the deuterium for two hours did not restore excitability in any of the five retinas. The simplest explanation to account for all the results is that structured dissociated water has a key role in excitable tissue and B-Z.

Figure 6(a) and **Figure 6(b)** show a type of qualitative change in optical properties of patches of retinas with sudden onset suggestive of a qualitative phase transition in the system dynamics.

Figure 6(a) shows a frame taken at the end of the first second after a short pulse of exogenous potassium (100 μl of a 200 mEq/l of KCl solution aspersed over the tissue surface with an Ependorf pipette). Note the half moon pattern and the small micro-lesion in front of it. This small micro-lesion is the one that split the wave shown in **Figure 2**. **Figure 6(b)** shows the scene 15 seconds later when a wave that was generated at the standing pattern is propagating as an isolated circular wave. The split and the irregularities of the initial shape are forgotten by the system. Note the shadow of the glass microelectrode in A. Its tip was removed from the place with the micro-lesion and now is covered by the time series records that sample the potential at the electrode tip and the optical signals in a square pixel matrix that is set at the surface of the tissue just over the electrode tip. The upper time series (predominant green color) with two peaks show the optical profile of an isolated circular wave (the wave shown in **Figure 2**). The lower time series (predominant yellow) show the extracellular potential drop (negativity is upward). As already stated above the peak of the dV/dt time series coincides in time with the peak of the dIOS/dt time series [78] or the potential drop and optical changes are synchronous but far from causal and linearly related in the mathematical sense [78].

This type of standing pattern has a persistence of tens of minutes and they can just disappear (the case in the experiment shown) or evolve toward a macro-lesion. The



(a) (b)

Figure 6. Two frames from the same experiment. Upper part of each frame shows the simultaneously recorded time series that shows the optical profile upper row (shown in green) and the extracellular potential drop recorded in the neuropil. The sampling rate was 10 HZ. The total time spanned by the time series is 20 minutes. The wave shown in the two time series is the wave splited showed in a previous figure (**Figure 2**). Note the two components of the optical profile and how the first component associates with the potential drop. The optical profile is made averaging the brightness of square pixel matrix (50 × 50 μm size) at the surface overlaying the electrode tip. The shadow of the electrode is seen in A but the tip region is covered by the time series records. (a): the frame was taken at the end of a second following a short pulse of a KCl solution (200 mEq/l) delivered with an Eppendorff pipette (100 μl total volume) close to the tissue surface. A patch of tissue (half-moon shape) shows the light scatter typical of excitated states. In front of it lies a small spot. This spot is the microlesion that split the wave shown previously (**Figure 2**). (b): 15 seconds later a solitary circular wave spreads covering most of the frame. This wave originated around the standing pattern shown in A. Note that the irregular shape of the origin and the split at the microlesion are "forgotten" by the system. The wave in B has 2 mm diameter. Note that the standing pattern is still in place. It lasted 30 minutes before fading away slowly. The frames have 8 bits of grey levels of brightness displayed in false colors: black, deep blue, pink, red, green, light blue, yellow and white.

analogy that comes to the mind is the image of a tropical storm watched from a satellite dish. The storms stay in place or move very little unless they become a hurricane. Using voltage sensitive dyes, we could image a macroscopic dipole standing pattern that shimmered in place [29]. The shimmer suggested fast vibrations at overlapping spatial and temporal orders of magnitude within the overall pattern.

Standing patterns are frequent at macro lesion borders and in such cases they are called "penumbra zones". The birth of such a pattern and lesion followed with the red scatter signal can be seen in [29] [72]. When such a pattern arose in a region in which one electrode was inserted, a potential drop of 4 - 5 mV was observed and the extracellular potassium activity went from 6 mEq/l (the solution concentration) to 10 mEq/l and stayed there while the standing pattern was present. In other words, standing patterns are immobile electrochemical patterns. They can give rise to solitary waves as shown in **Figure 6**. By contrast, they themselves are obstacles to wave propagation and split waves [78].

In summary: lipid bilayers are a complex system themselves, although compared to biological membranes they are drastically reductionist models. Nevertheless, they made

and still make a contribution to the understanding of physiology of membranes. Their phase transitions are not usually discussed in this context, and we think they should be. To understand excitable membranes it appears that volume phase transitions in the external leaflet and lipid bilayers phase transitions will have to be combined. Last but not least, when interacting membranes at two and three dimensions are concerned, then qualitative emergent changes in the tissue dynamics can appear. The standing pattern shown in Figure 6 is just one example. Evolving spiral waves either solitary or with two or three interacting spirals also were observed in retinas and hence can be predicted in the heart as well (scroll waves) [79] [80]. The self-sustained activity could have duration up to one hour.

Figure 7 shows the complex spatiotemporal pattern of an evolving solitary self-sustained spiral experiment in chicken retina [80]. The pattern shows the position of the maximum curvature (and hence the minimum spread velocity) of a spiral wave. This is the point in which the wave turns. It reflects either a particular state of that patch of tissue or a set of states compatible with a low excitability state. It is a beautiful pattern in which the great organizer is time.

Time as the global coupling agent will imply irreversible thermodynamics as the

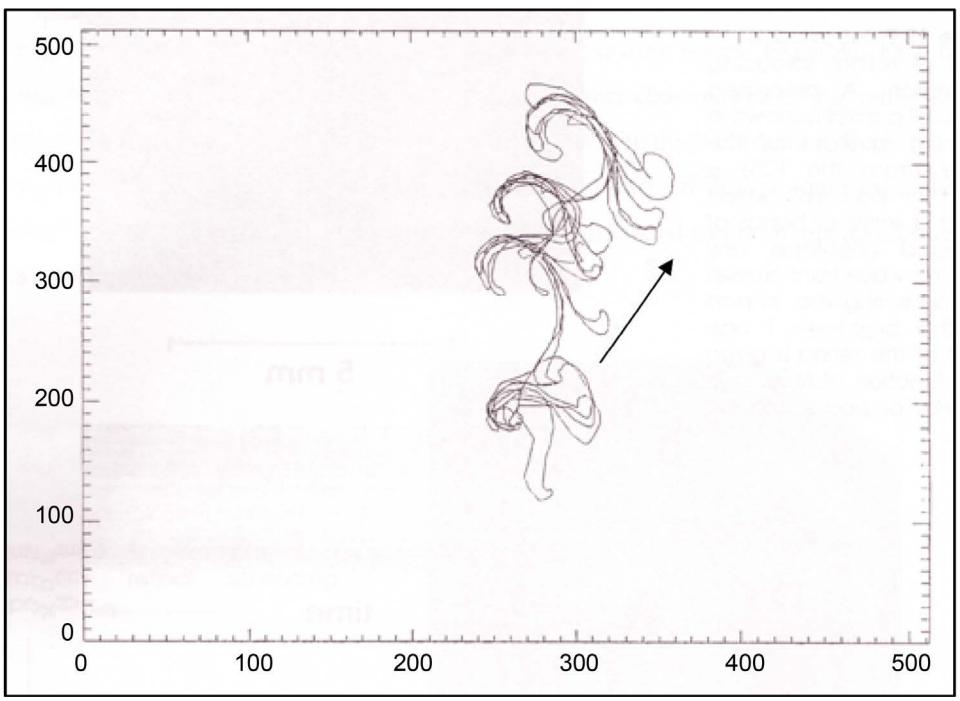


Figure 7. The low dimensional pattern was obtained projecting in two dimensions of space the temporal evolution of the site with the maximum curvature of an evolving self-sustained spiral wave of retinal spreading depression. The arrow shows the time direction. The pattern shows the drift of the turning point of a logarithmic spiral wave. Note that what drifts is a dynamic state of the system. The arrow show how the pattern evolved in time. Modified from [80]. The image from the camera was digitized in 512 pixels in both X and Y axis. Because of the camera ratio of 4/3 (X versus Y), 1 mm equals 170 pixels in the horizontal axis and 102 pixels in the vertical axis. In the experiment shown in the figure, a single spiral completed 18 revolutions in 45 minutes.

theoretical context adequate to explain macroscopic electrochemical patterns in tissue.

5. Direct and Circumstantial Evidence for the Polyelectrolyte between Membranes Role in High Mental Functions or the "Smart Gel" Hypothesis

At the introduction section we stated that the belief that quantum mechanics in the form of the Schrodinger equation could explain high mental functions had no validation from experimental results. In this section we will make the case for the irreversible thermodynamics.

We begin with the belief that only the neurons in the brain are the elements responsible for perception and learning mechanisms. This belief is the direct descendant of the "neuronal doctrine" of Ramon y Cajal, he was a master of the histological Golgi technique of staining central nervous system. By contrast, Golgi himself, the creator of the method saw a system of network relations, a much more anarchist brain in contrast to the single units, hierarchic brain saw by Ramon y Cajal. In here we will defend the belief of the irreducible complementary unit of the glial and neural networks and the fact that they interact through the polyelectrolyte gel of the extracellular matrix. A good example of this view is the review of Robert Galambos of 1960 [81].

We own to Ichigi Tasaki (1958) the discovery that glial cells contribute to slow potentials recorded at the cortical surface [82]. In the early sixties of the last century (1964), two slow potentials were discovered, the "readiness potential" and the Contingent Negative Variation (CNV) [83] [84]. Both potentials signal preparation for behavior in humans. If we assume that the mechanisms at central gray matter are the same, then results from the retina can be extrapolated to the cortex. In retinas there is ample evidence that slow potentials are dominated by glial membrane depolarization [85]. The "b-wave" of the electroretinogram is concomitant with glia depolarization and increase of extracellular potassium activity. In the retina, the dark and light adaptation are also concomitant with increases and decreases of extracellular potassium (the retina sensitivity range is over 6 log units of energy input) [85]. Therefore, the standing patterns we see in retinas can be just an enhancement of physiological mechanisms of excitability control; and functional syndromes as the "petit mal" generalized seizures and hallucinations of temporal seizures could have such electrochemical reversible patterns as the pathophysiological factor underlying the symptoms.

The visual scotomas that propagate during the visual aura represent a distortion of perception. Observing his own scotomas, Lashley [86] concluded that a wave of excitation/inhibition travelled in the primary visual cortex at 3 mm/min. Today, the idea that spreading depression waves can explain the migraine aura is more accepted in medicine. The standing patterns we shown are also candidates for perception distortions or hallucinations, even the "transient global amnesia", could be explained by vanishing standing electrochemical patterns in the CNS.

There are several decades of studies showing that spreading depression waves impair memory and block learning (see for example [87] [88]. On the other hand, we have the

report of secretion of an extracellular acidic glycoprotein associated with learning in fish and rodents [89]-[91]. This protein, ependymin, configuration depended on calcium activity in the extracellular matrix. With low calcium levels, ependymin formed polymers within the matrix. Last but not least, it should be noted that Alzheimer dementia histopathological findings are characterized by alterations of the extracellular matrix the so called senile plaques (besides the intracellular tangles inside neurons). This senile plaques are heteropolymers that contain, among other components, a part of an intrinsic membrane glycoprotein, β protein and glycosaminoglycans (GAGs). Even before the appearance of the full plaques the presence of the β protein in the extracellular matrix can be detected together with impaired learning [92]. We have seen the power of protamine interaction with the basement membrane of retinas: excitability collapse, probably due to heteropolymers with heparan sulfate, a GAG [33].

Back in 1971 [93] Neumann and Katchalsky predicted a possible role of the polyelectrolites associated with membranes as a memory mechanism, we quote… "Controlled changes in the environment of metastable macromols or subcellular macromol, organizations such as membranes by high elec. fields or by ion gradients can induce conformational changes which could serve as reproducible imprints of a memory nature." It turned out that their prediction was fulfilled in 1977 with experiments with the basement membranes of striated muscle. A result confirmed many times [94]-[97]. In muscle it is possible to cut the innervating axon and to kill the muscle cell leaving intact the surrounding basement membrane. If either the muscle cell or the axon is allowed to grow, both will create the membrane proteins typical of the nerve/muscular junction at the exact location of the previous one. In other words, the pattern of the lost innervations is stored in the basement membrane for at least two weeks. Prediction fulfilled. The obvious consequence is the strengthening of the Neumann and Katchalsky conjecture about memory mechanisms.

If we take into account that the only direct evidence for microtubules storing energy is a misinterpretation of results—the energy measured was the interfacial water energy—then the case for the polyelectrolites that fill up the named extracellular space has first, a prediction fulfilled and after that, much more direct and circumstational evidence for its case. The polyanionic gel that conveys the glial neural interaction in the CNS could be the smartest gel we know. We have to grant to Stuart Hameroff, the adherence to his beliefs. In his 1982 paper [7], he mentions that there was evidence of microtubules involvement in anesthetics response mechanisms. Well, we think that the approach of Tomas Heimburg, that physics suggests a bilayer primary effect of anaesthetics, agree with classical physics and there is no need of nothing else. In one aspect of Hameroff and Penrose [8] approach to mind we agree: It is possible that perception and memory is an energy pattern instead of a material structure. Observing electrochemical patterns with red and infrared cameras, what one sees is the dynamic changes in potential drops or energy release.

Another prediction made by Katchalsky were coherent flow of matter or energy in polyelectrolyte systems and the coupling of electrical and mechanical energy in these

systems that could be used by living cells and tissue [19]. At the end of Section II we mentioned a simple heart model made of a B-Z reaction system coupled to a pH sensitive gel. A recent publication in this research line shows very clearly both of Katchalsky predictions in attached movies. Because we primates believe in what we see, the doubtful reader could take a look at the experiments of Miyu Ioshii et cols. (2016) [98].

We will finish mentioning two publications of Arturo Solis Herrera that defends that energy from split water molecules made by melanin is an important source of energy in the CNS [99] [100]. If he is correct, then the pecten would be strategically posed in avascular retinas as a source of energy directly from light. It appears that we still have much to learn about water and living beings. We found a quote from the Upanishad in a paper about the state of water inside cells [101] that we reproduce here: "***It is water that assumes the form of this earth, mid-region, this heaven, these mountains, these gods and men, cattle and birds, herbs and trees, and animals together with worms, flies and ants. Water indeed is all these forms. Meditate on water***".

6. Conclusions

It is a forgone conclusion that physics and mathematics will have to explain all phenomena in biology. The question is which branch of physics to use. The reality of time and its central role in irreversible thermodynamics make it in our opinion the adequate branch to explain biology. Neither classical nor quantum mechanics have a role for the arrow of time.

If agreement with experimental results, fulfillment of predictions and integration of apparently independent results are relevant criteria in order to support or refute a conceptual framework, then irreversible thermodynamics again appears the adequate background to test self-organization in excitable tissue. By contrast, the paucity of direct experimental data, and the alternative interpretation of these data [9], do not support relevance for quantum mechanics in explaining brain self-organization, or even worse, high mental functions. They, (predictions and results) reinforce the role of structured and dissociated interfacial water as both, a store and coherent releaser of electromagnetic energy.

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