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Reversibility of excitation waves in brain and heart and the energy of interfacial water. Can reversibility be explained by it?

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ABSTRACT

In this manuscript, we interpret the implications of a discovery we made in 1993 for the understanding of the spread of excitation waves in axon, central gray matter (isolated retina) and heart. We propose that the initial burst of energy dissipation in these waves measured as potentials drops, ionic activities marked changes or optical properties being mostly the effect of dissociated water becoming liquid water and be reversible due to the further on dissociation during the refractory period. We also propose experiments in order to falsify or agree with this conjecture.

1. Introduction

In 1993 we made a (serendipitous) discovery: using two cameras, with different optical filters in front of each, we examined the initiation and propagation of two dimensional waves (well almost or *quasi two -dimensional*) in central gray matter (CNS). We observed that the red and blue components of the light scatter measured in the waves told different stories as far as time of onset and spatial frequencies (lower frequencies and later onset of the blue part). Intuition led us to believe that was an important finding and we published the observations in several papers, two in mainstream neuroscience literature (Fernandes De Lima et al., 1994; 1997a, 2001, 2002).

The review of 1997 was the last paper we published in mainstream influential neuroscience journals, from there on, we were banished and worse than being criticized, we were ignored (one will not find any reference to our work in all reviews about spreading depression or brain functional syndromes). On the other hand, we could publish in physical chemistry and experimental chaos journals. Thus, to our disappointment (we did our best, it was not enough…) the finding was ignored by the neuroscience community. However, we kept going observing three excitable media, artificial lipid bilayers, the retinal spreading depression and excitotoxic responses in retinas and the B-Z (Belouzov-Zabotinsky) reaction system. A turning point happened in 2008 as far understanding the red scatter of light at wave onset: we found that the substitution of water by liquid deuterium led both the *in vitro* retina and the B-Z system to collapse of excitability in a very short time. The result was unexpected and very exciting. The idea was to change a factor of the global coupling in both systems, the solvent, and look at the effects.

The physical properties of deuterium solutions differ from those of water solutions, as discussed by John Katz in 1960 (Katz, 1960). Liquid deuterium and water have a similar dielectric constant and surface tension. By contrast, the viscosity of deuterium at 25 °C is 25% greater than that of water. The temperature of maximum density (g/cc) also differs: 3.98 °C for water and 11.2 °C for deuterium. This difference can make the liquid deuterium effects similar to cooling or apparent temperature effects in experiments. However, the greater difference (except mass) between the isotopes is in self-ionization: liquid deuterium's self-dissociation is five times smaller than that of water, or its pH at 25 °C is 7.41 instead of 7.0. These results were shown as a poster in a meeting of the Experimental Chaos Society in Sicily in 2009 and later published in two separate papers[1]- (Fernandes De Lima and Hanke, 2011; Klink et al.,

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2010). Only one conclusion was possible, the physical properties of the solvent in both retinas and B-Z was crucial to the systems excitability. The first question to be asked was: Can water store charge? The answer was yes (Ovchinnikova and Pollack, 2009).

One of us contacted Gerald Pollack and through him we became acquainted first the existence and second with the optical properties of dissociated interfacial water (Zhengh and Pollak, 2003; Zhengh et al., 2006; Chai and Pollak, 2010) and (Bunkin et al., 2013, 2014, 2018). Very recently, Nikolai Bunkin group published a paper on the effects of liquid deuterium on the Nafion interfacial water or EZ (Exclusion Zone) water (water at the hydrophilic polymer surface and bulk liquid water). As we predicted, the width and stability of the EZ water were deeply affected by liquid deuterium (Bunkin et al., 2018) just as action potentials, retinal waves and B-Z systems (see below).

Because the implication of dissociated water means energy involvement, we changed our minds about the interpretation of the uncanny parallel qualitative changes we observed in bilayers, B-Z and retinal waves responses to electromagnetic and gravity fields (Fernandes De Lima et al., 2001; 2002; Hanke et al., 2001; Wiedemann et al., 2010). Before the liquid deuterium experiments, we attributed the parallel similar behavior of both systems to the mathematical identity of the equations used to describe their dynamics, if we use the Fitz-Hugh Nagumo action potential model and not the Hodgkin Huxley one for the central gray matter excitation waves and the Brusselator for B-Z systems.

However, we only could link our early discovery of the meaning of the red scattered light at the wave onset or its propagating forefront, when we watched the videos and read the papers of Elmar C. Fuchs on the water bridges[2]. The link between dissociated water dissipation of energy and excitable media we will discuss in the next section.

Water and its resonances- it is common to teach in high school that the ideal pendulum “stores” potential energy and transforms it in kinetic energy. The ideal pendulum in truth does not stores but transforms one energy form into the other energy under the force of gravity. The pendulum is a system that has the ability of energy transformation, the same is true for any resonator: the dissipation of energy overlaps the energy transformation.

Watching Elmar C. Fuchs videos with water bridges, we realized that these bridges are very useful experimental model of dissipative structures (as defined by Ilia Prigogine) far from equilibrium. He made infrared cameras images of the bridges and used classical Maxwell theory to explain their behavior. What was somewhat surprising was the very small but stabilizing effect of gravity in explaining the steady state. However, classical electromagnetic theory cannot predict or explain the oscillation at the onset of dissipative structures, or the sudden qualitative change when the bridges just form. Furthermore, his measurements of infrared dissipation within the bridges brought another surprising result, two wavebands. The bridge is too small to have two temperatures, as dissipation would level these out; therefore, one wave band had to be non-Plank infrared dissipation of energy. Mechanical flow within the bridge and between the two beakers used to apply high voltage to feed the bridge, are the other forms of energy dissipation by liquid water in bridge experiments.

Water is a master in absorbing any form of energy and immediately dissipating it in coherent fluid flow, infrared light and heat behaving as an amplifier and resonator with similarities with a LASER system dissipation of energy[5] (Fig. 1).

One of the best devices to observe low dimensional dynamic water patterns are John Stuart Reid[3] experiments on liquid water resonances. In principle, such a set-up mechanically (loudspeaker, piezo-actuator) vibrates water, with the sound introduced into the device by an amplifier (see also cymascope.com). In Fig. 1 we show the development of the 3D pattern of resonance of deionized liquid water to an acoustic input of a single frequency (a piano key stroke). We can see the resonant 3D structure because the optical properties of the water in the coherent flow (coupled behavior of water flow) differ from the bulk water. A possible interpretation is that this change is due to the quasi-liquid crystal properties of dissociated water, analogous to what one sees in a water bridge, dissipation of energy (Elmar Fuchs). In Fig. 2 we show the 3D patterns obtained in a resonant crystal dissipating light energy. The similarity is uncanny and one can only speculate what nature is telling us. Note that in the LASER patterns only electromagnetic energy flows while in the cymatic pattern matter flows. Last but not least, it was observing the videos of John Stuart Reid that we could visualize the superposition of resonances at different scales of space and time predicted by Katchalsky in the late 60th as a key factor in understanding excitability in biology. In the complex dynamic structure shown, coherent flows at several scales are superposed in order to create the complex resonant structure.

Resonant cymatic patterns as shown in Fig. 1 and water bridges are both examples of dynamical dissipative structures. Dissociated water close to polyelectrolytes surfaces or EZ zones are another. In this text, we assume the EZ resonances very relevant to biological systems.

EZs have a different refractory index from bulk liquid water, as measured by Nikolai Bunkin et als, (2013, 2014). Here we are assuming that the drifting water within the coherent flow of sound dynamic patterns, is again dissociated water, analogous but not identical to the EZ dissociated water. This is because at the polyelectrolytes interfaces close to the Nafion surface and in biological membranes we have ionized COO^{-} and $SO3^{-}$ radicals typical of the charged gels named glycocalix and basement membranes. The interfacial water in EZs is dissociated water.

Distilled or deionized liquid water make the sound patterns and the water bridges. All three (cymatics vibrations, bridges and EZs) dissipative structures have a sudden onset (seconds) and long life steady states of energy dissipation.

As far as we know, the images shown in Fig. 1 is from a black white camera, not an infrared one. It appears that the water optimizes the dissipation of the mechanical energy imposed in the system, just as the water in the bridge does with the imposed electrical energy. If the EZ water and the sound pattern shown in the figure are analogous as we assumed, them the substitution of water by liquid deuterium would also show a short lived and more disorganized sound pattern, the same effect deuterium had on Nafion EZs (Bunkin et al., 2018), retinal waves and B-Z systems. Certainly a feasible experiment in very convenient space and time scales. If the liquid deuterium fails to change the 3D resonant cymatic pattern, falsifying this conjecture, categorically.

Elmar Fuchs noted that free protons and hydronium ions would have different flow velocities, as well the HO^{-} anion, but there is no word about the free electrons flow. The emphasis of the classical approach is energy conservation whereas in the dynamic dissipative structures of interest for biology, energy transformation is the key factor. We know that at Nafion EZs, electroneutrality is gone and the EZ is negatively charged. The same is true for biological membranes, charged at the interfaces. The EZ potential can be very large (124 mV) and its width as large as 100 μm; a scale several orders of magnitude larger than water polarized molecules in bulk liquid water.

Nature appears to give us experimenters the ideal preparation to observe biological EZs resonances. It is the isolated anterior chamber of the vertebrate eye (Fig. 3). When the eyecup of young chicks is cut at the equator, the anterior chamber plus a ring of sclera is accessible. The sclera, glued to a dish, will create several interfaces that will be available to look for resonant 3D patterns.

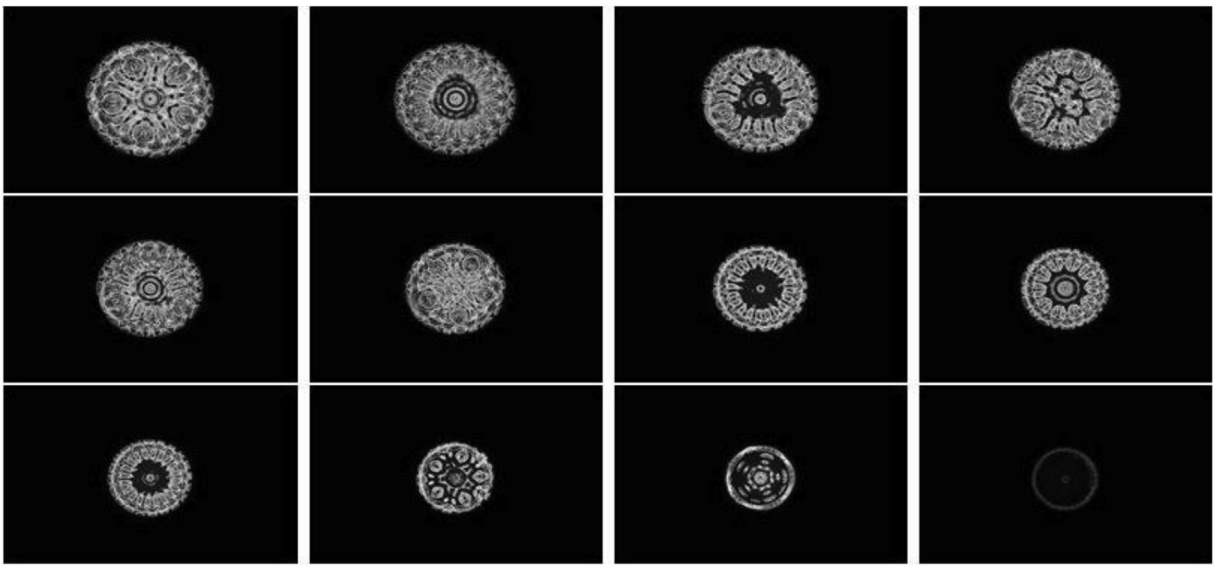

Fig. 1. 3D low dimensional patterns observed in liquid water responding to the stroke of a piano key. Intensity decreases from the first to the last frame. These patterns always begin with a single torus near the interface water crystal of the cymatic device created by John Stuart Reid, then several tori appear until a third fast jump shows the complex pattern shown in the first frame. Modified from www.youtube.com/watch?v=9al397N6Tzs.

Fig. 3 show these interfaces schematically: the lipid interface with monolayer of amphiphilic lipids between air and liquid tear. Then comes the transition between the charged gel closed to the epithelium and the liquid. It appears to be the ideal preparation to put on a cymatic device and observe resonances. The branching molecules are mucins, glycoproteins in which 85% of dry weight are the sugar residues rich in sialic acid (COO^- radicals), hyaluronan (COO^- radicals) and SO3 radicals. The mucins are integral parts of both ocular epithelia from cornea and conjunctiva making the external leaflet of their membranes; and thus, at least conceptually, the ideal experimental preparation to observe epithelia EZs in action. It should be noted that the physiological concentration of reduced glutathione (GHS) is 5 mM and thus the maintenance solution must include at least this concentration of GHS otherwise the preparation will be short lived, because the illuminating light creates H_2O_2 and the free radicals will kill both epithelia very quickly.

1.1. Some reflections on the reversible aspect of waves in excitable media

Action potentials (AP), spreading depression (SD) waves, and waves in the Belousov-Zhabotinsky (BZ) reaction system belong to a general class of propagating waves in so-called excitable media. Such systems have to fulfill some basic requirements: among others they have to be open, they have to be far from equilibrium, and feedback and non-linear interaction must exist between the components. In open systems, the entropy can become smaller without violating the second law of thermodynamics, as entropy is decreased by using energy. Even in case the loss of energy by dissipation and heat production, during propagating waves this will be compensated as long as sufficient energy is given or added to the system.

The classical view is that in cells and axons generating and propagating action potentials, the energy is stored in the ionic gradients, created by ATP consuming pumps. Taking for example an isolated giant axon and pharmacologically cutting ATP synthesis. This axon will be still able to allow propagating action potentials until the ionic gradients become too small, in fact, this might be 1000 or more AP's, since the net ion movement during an AP is small. Nevertheless, finally, no action potentials will be possible any longer. Thus, on a longer time scale the system is no more reversible.

Looking at the spreading depression, the situation is somewhat different. At the wave front the ion-gradients are collapsing significantly, and thus in a later state must be reestablished by ATP consuming pumps. This kinetics follows the second phase of the IOS in retinal spreading depression waves. Blocking ATP synthesis in such a system, one wave can still propagate through the tissue but at its backside the tissue will die, as has been shown in numerous experiments. Accordingly, in nervous tissue without ATP production, reversibility is no longer present for spreading depression waves.

Finally, in the B-Z chemical system, the energy is chemical energy mainly in oxidation processes. This can be easily observed in the oscillating B-Z, which will stop after some time in a system without delivery of new material. Again, the process is not reversible on a longer time scale. Experimentally this can be overcome by creating a continuously flow reactor system permanently delivering new chemicals and taking out of the system end products of the ongoing chemical reaction.

Accordingly, it can be stated that all three systems are reversible given the following definition "a process is reversible when the beginning and end state are identical". This is not precisely identical to another definition of reversibility saying that a process can run in both directions in space and time, or that transfer of energy happens in both directions. However, as long as sufficient energy is present in the system, waves in excitable systems do not violate the second law of thermodynamics; see above. However, some problems with our definitions may be obvious when stating that all three processes can be described by a simple three state model – excitable, excited, and refractory. From the refractory state, the loop back to the excitable state must be closed. Using a classical physical definition, this is a thermodynamical cycle. The process (the wave propagation) always starts at the excitable state, the question might be asked; can it start at the refractory state and run backwards - obviously not. This question furthermore is related to the statement that colliding waves should annihilate each other in systems following the above given definitions. A nice approach to this

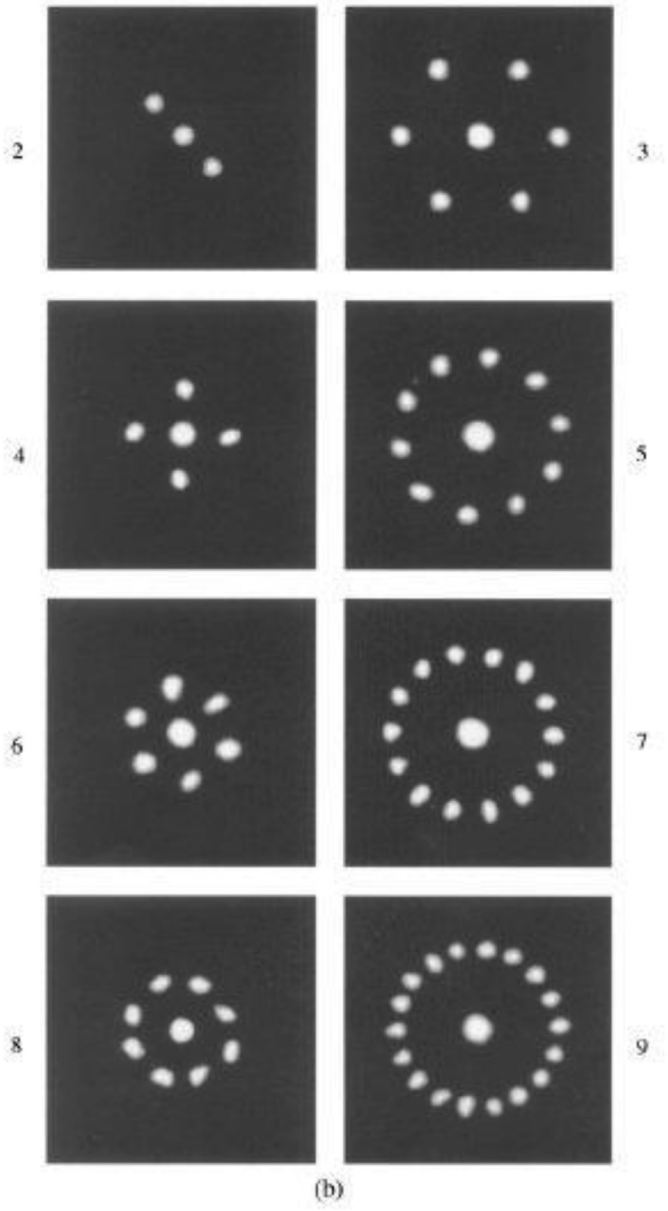

Fig. 2. Laser system with quasi liquid crystal behavior in low dimensional patterns in a Kerr medium (refractive index of the medium is depending on applied potential). Far field or global coupling effects are emphasized instead of near field or local coupling effects. Modified from (Arechi et al., 1999).

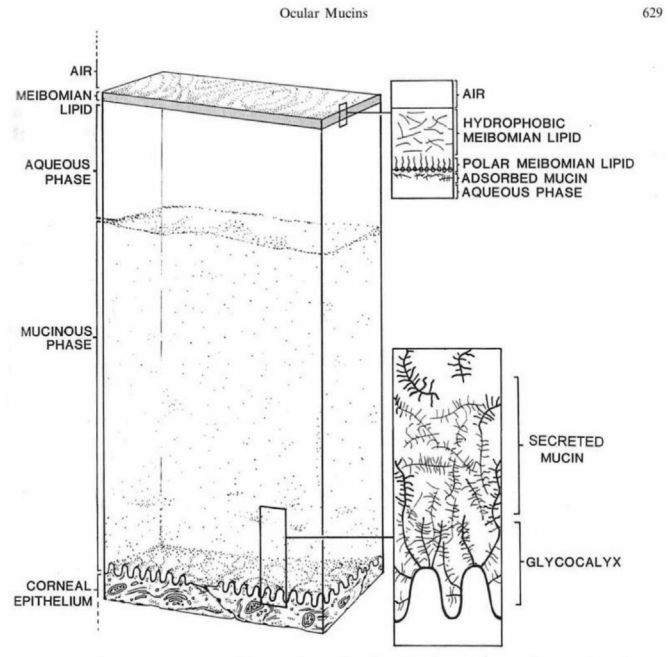

Fig. 3. Schematically depiction of anterior eyecup interfaces: the charged gel contains integral membrane mucins from cornea and conjunctiva epithelium plus secreted mucins mostly from conjunctiva goblet cells. The chick eye has a very convenient spatial scale in order to observe EZs biological 3D resonances in cymatic devices. Modified from Corfiled et als, 1997 Prog. Eye ret. Res., 16(4); pp.629.

question is to play with cellular automata. In an automaton with only two states, live and dead (see i.e. Conway's game of life) collision without annihilation is possible, in an automaton with states including graded refractoriness (see also (Adamatzky, 2002, 2004): and Peixoto (1997) for cellular automata model of retinal spreading depression (Peixoto, 1997) collision brings annihilation.

Related to action potentials, the mainstream approach clearly states that these annihilate upon collision, but there is still some controversy in the literature (Mimura et al., 2002; Fillafer et al., 2017; Gonzales-Peres et al., 2014; Berg et al., 2017). This discussion includes the question whether the refractory period is responsible for the behavior of traveling pulses (Tasaki, 1949). In B-Z waves the situation again is not clear, upon collision wave fragments can annihilate, fuse, split, or deviate from the original path (Adamatzky, 2004). Additionally, it has been postulated, that reaction-diffusion waves of the FitzHugh Nagumo type may reflect off before they collide, this being again in contrast to annihilation as found in AP's and B-Z (Mimura et al., 2002). In spreading depression research not very much has been published related to the question about collisions, but at least it has been indeed shown that SD wave annihilate upon collision (Shibata and Bures, 1972) and that waves speed up before they collide (Goldermann et al., 1998), this finding being not yet fully explained.

Finally, the question has been brought up, whether there is a biphasic temperature change correlated with a propagating AP (Tasaki, 1982, 1999) and similar wave propagation processes in CNS (spreading depression waves) (Tasaki, 1982, 1999) and similar wave propagation processes in other systems, which is correlated to ask whether AP's propagate with or without dissipation. Finally, Tasaki in his 1999 paper stated the importance of water molecules and Ca^{++} in the process of nerve excitation and that there is evidence for phase transition in nerve fibers related to propagating AP's.

Putting together everything tells us, propagation of waves in excitable media is reversible to a certain content, but accompanied by dissipation of energy to heat and conversion of energy to a transient and local decrease in entropy. This matches the second law of thermodynamics. Irreversibility takes over when the energy reserves are consumed. Absolute thermodynamical reversibility, as is defined by physics according to our knowledge has not yet been shown in any of the wave propagations as there are AP's, B-Z or SD-waves. At least following our interpretation always there is consummation of energy in excitable media.

Phase transitions at the wave front and changes in the structure of water are mechanisms being involved significantly in these wave propagation processes in all three systems, and are under discussion.

The optical concomitants of nervous system excitation waves and their coupling to ionic activities changes- Fig. 4 shows the typical, meaning more frequent, temporal evolution of ionic

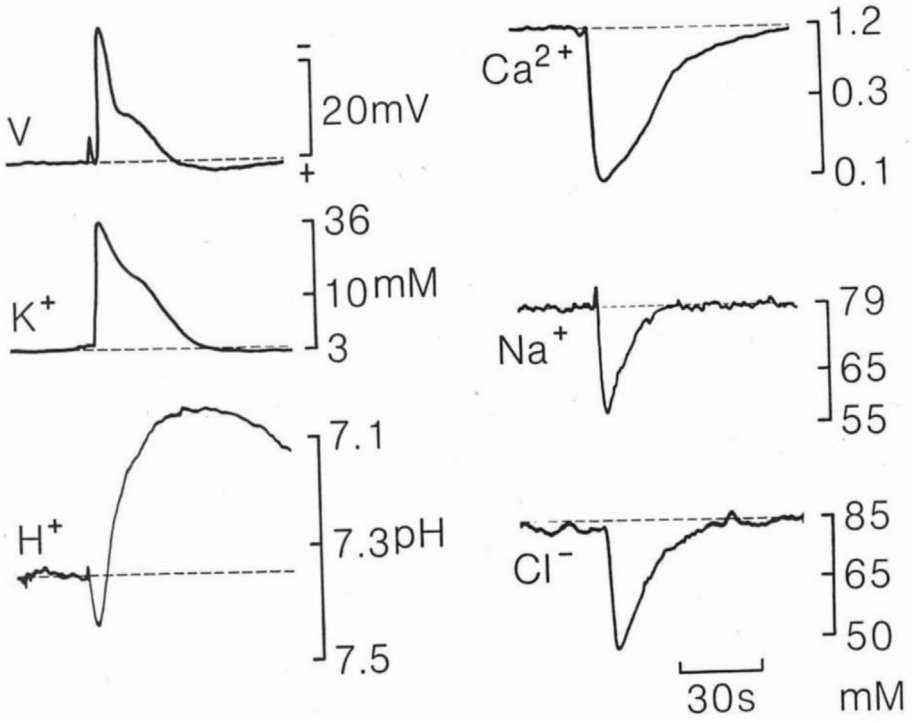

Fig. 4. Extracellular potential and ionic activities changes associated with the wavefront of spreading depression waves in central gray matter in this case, cerebellum. Note that the potential drop is shown upward for historical reasons. Note also the large oscillation preceding the abrupt potential drop. All curves represent the typical or more frequent outcome at wavefront of excitation waves in brain. Modified from Kraig et al., 1983.

activities changes measured at the wave front of spreading depression waves in cortices, cerebellum and retinas. The amplification of electrical potential oscillations shown in the temporal evolution of extracellular potential before the transition from quiescent to excited states is a less frequent event in the gray matter waves. This negative shift is associated with increases in extracellular potassium activity (Fernandes De Lima et al., 2001; 2009). In this section, we will discuss the tight coupling between pH changes and optical signals.

The alkalization observed in the extracellular gel is difficult to explain with the classical membrane theory that assumes the extracellular gel as diluted solution of ions that cross the membrane along activities gradients via channels and against these gradients through “pumps”. We cite…“The observation that glia initially become more alkaline during electrical activity is thus paradoxical. The correlation of glial alkalization with evoked electrical activity suggests that modulation of intracellular pH of glia may have important functional implications” … (Kraig et al., 1983; Chesler and Kraig, 1987, 1989). Well, we agree with the authors, one will have a hard time to interpret the result if metabolic origin of the pH shift is assumed. By contrast, if one assumes that dissociated water becomes bulk liquid water, then it is easy to understand; provided that this alkalization is observed tightly coupled to a concomitant loss of birefringence in excitable membranes. Figs. 5 and 6 show the Intrinsic optical Signal (IOS) measured in isolated axons (it should be noted that isolated axons are not separated from the glia in myelinated or non-myelinated -Schwan cells- fibers and even in this case more than one membrane is involved in the optical measurements) when an action potential is elicited (Cohen et al., 1968). The alkalization measured in cortices, isolated cerebellums and isolated retinas (Casper et al., 1987; Ferreira Filho and Martins-Ferreira, 1992) appears a general phenomenon.

The assumption we take here is that action potentials waves are

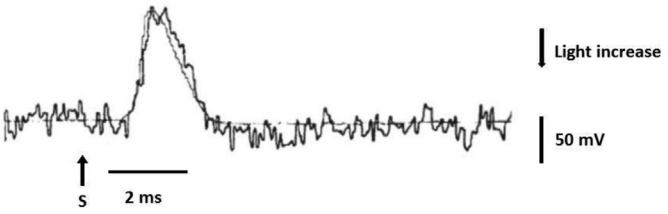

Fig. 5. Light scatter and potential drop (shown upward) recorded simultaneously in isolated giant axon of squid. In this figure the sick trace shows potential and light scatter at 45^0 is the light trace. S is the electrical stimulus. Modified from (Cohen et al., Nature, 1968). In this case excitation is tightly coupled to a decrease in light scatter.

excitation waves in one dimension, spreading depression waves in isolated cerebellums and retinas the same phenomenon in two dimensions and cortical spreading depression and the heart beat excitation waves in three dimensions. The geometry of the glial network the key determinant factor (see Fernandes De Lima et al., 2014) in waves in central gray matter.

In Fig. 4 the extracellular potential curve show a macroscopic oscillation in the extracellular potential preceding the wave; and, when present, this oscillation is associated with increase in extracellular potassium activity (Fernandes De Lima et al., 2009). The maximum rate of change in potassium activity coincides with the maximum rate of change in the potential, the calcium activity change initiation coincides with the maximum rate of change of potential and potassium (Fernandes De Lima et al., 2009; Somjem, G.G., 1993). The classical view is that ions move in and out of cells, however, ions activity change if their screening of charged gels changes, the measurements with ion sensitive electrodes cannot distinguishes between these mechanisms. Calcium activity is specially affected by this ion screening of charged gels (Katchalsky, 1968). Katchalsky estimated that 99% of calcium was bound to

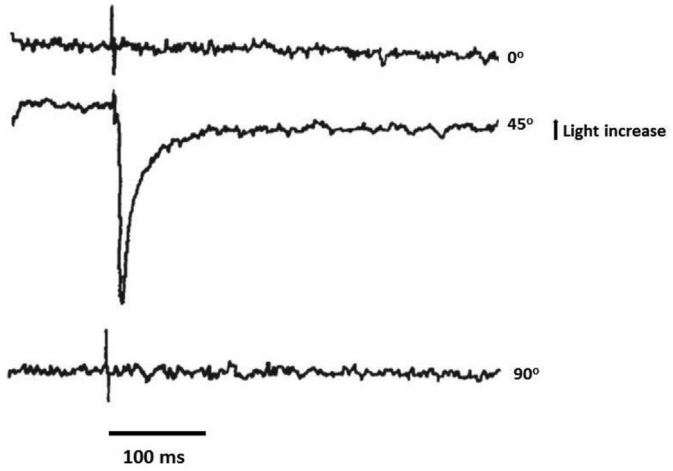

Fig. 6. The traces show the fall in birefringence in an isolated giant squid axon during one action potential. Note the difference in time scale from previous figure. Suggesting a sudden structural change with a much slow relaxation or recover. Modified from (Cohen et al., Nature, 1968).

alginate polyelectrolyte.

The Intrinsic Optical Signals (IOSs) consists in changes in light scatter (Tasaki, I., 1999, Martins-Ferreira e Oliveira e Castro, 1966, 1971; Oliveira e Castro and Martins-Ferreira, H (1970)). The most frequent outcome is an increase in light scatter that in retinas changes the degree of transparency- more than 90% of measurements will show this increase-, however, we choose to show that decreases in light scatter are also measured during excitation (Fig. 5). The tight coupling between the potential drop and optical changes is obvious in Fig. 5. In retinas, the change in light scatter changes with physical chemical properties of the maintenance solution. For example, increasing NaCl from 100 to 120 mEq/l the optical profile will show a hyper transparent ring before the light scatter increase. Increasing NaCl further to 140 mEq/l, makes the retina loose transparency and the waves IOS changes to the type shown in Fig. 5 or "dark retinal waves"[3].

In Fig. 6 the loss of birefringence of axons is shown; note the slow recovery and sharp onset. Compatible with phase transitions in the gel that makes the external leaflet of excitable membranes. These phase transitions called "volume phase transitions" (Tasaki, 1999, 2008) in which the charged molecules in the gels change their relation with water and screening ions; consequently, structural changes take place abruptly within the gels. Followed by a slow relaxation back to the charged gel structure compatible with what we see: loss of birefringence associated with potential drop and alkalization due to hydronium ions and free protons reverting to liquid water.

In isolated retinas, birefringence has not been measured; an experiment waiting to be made. Martins Ferreira and Oliveira e Castro (1970) demonstrated that cutting the retina horizontally, separating inner from outer retina, did not impaired its excitability nor changed the IOS and the hydroionic concomitants. They concluded that membranes were the place of origin of the Intrinsic Optical Signal, we confirmed their results (Fernandes De Lima et al., 2014). The major part of the inner retina (i.e. the part closer to the vitreous border) was the place of the maximum of ionic changes and optical signal. The reduced retina preparation consists of fine tubes of glial cells devoid of large particles like the mitochondria and synaptic membranes both with a high surface to volume ratio. The avian central retina is flat and therefore in principle one can measure birefringence loss at excitation onset. Therefore, our interpretation of the optical changes can be falsified by

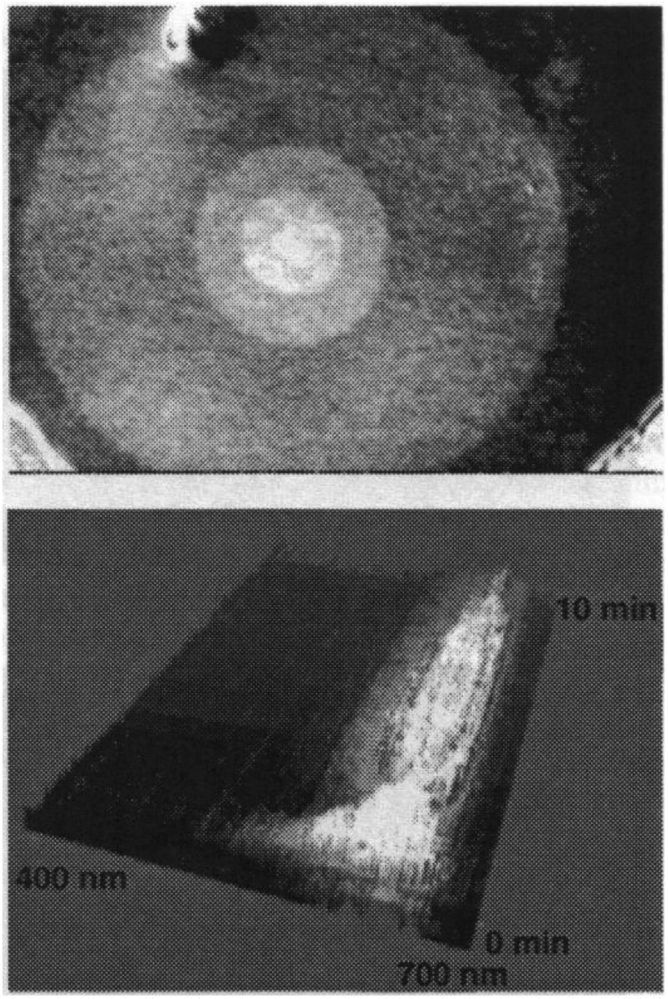

Fig. 7. Upperpart, fake "target pattern" created by summing up three frames of solitary circular retinal propagating wave and then subtracting a background frame. The bright white and black structure at the upper border of the frame is called pecten and in front of it lies the optical papilla, the place where the axons of the optical nerve come together and leave the eye. The circles show size at 10, 20 and 60 seconds propagation. Bellow: time/frequency spectrum of a circular wave. Frequency is in logarithm scale, time and amplitude linear scales. The total time is 10 minutes and thus the two components of the optical profile of a circular wave can be seen. The peak of the first scatter component is at 545 nM, the second component is more flat. Modified from (Fernandes De Lima et al., 2001).

experiments.

On the other hand, if the tight coupling of the optical changes and early alkalization of both intra and extracellular leaflets of glial membranes is just a coincidence is also very unlikely. Volume phase transitions of mucins (see Fig. 3) have been documented and are dramatical: a 600 times increase in volume in about 5 seconds after the release from goblet cells (Verdugo, P. 1984). The pH estimated inside the goblet cells is very low, around 3, and there is calcium whose role appears to be compacting the mucins by screening charged radicals or acting as counterion.

In Fig. 7 we show the propagation of a solitary circular excitation wave (spreading depression wave) in retina (Martin-s Ferreira and Oliveira e Castro, 1966, 1971). The details of the optical profiles of such waves were published recently (Fernandes De Lima et al., 2014). Below, the time/frequency spectrum of scattered light by the tissue. The first component or optical peak is the one coincident with the hydroionic changes observed in retinas, cerebellums, cortices and axons. The maximum is at 545 nM. The second

component has no clear frequency preference and coincides with production of lactic acid by glia and its secretion out of glial cells (see below). In Fig. 8 we show the effect of addition of glycerol on the retinal maintenance solution. Glycerol is a polar liquid and Elmar C. Fuchs showed that it also makes bridges analogous the water bridges showing that the difference in viscosity played no role in the capacity of bridges formation[2]. On the side we show the effect of glycerol on the potential drop recorded in retinal waves, the amplitude fell from 25 to 10 mV and the kinetics was altered showing a plateau followed by slow recovery to different baseline. This effect was observed in 50% of the waves, in the other half, the shape and amplitude of the potential drop did not change compared to the controls.

The field potential measurement appears to have a strong bias to local events whereas the structural changes associated with optical signals either have a distance effect or are due to **phase transitions** in the bilayer. An analogous interpretation is possible to explain the binary changes in light scatter at wave onset in axons and retinas. The difference is that according to Ichiji Tasaki, **volume phase transitions** in the glycoproteins and glycosaminoglycans associated with the external leaflet of membranes are also included in order to explain the optical and ionic activity changes (Tasaki, I. 2008). The external leaflet of neuropil (region where synaptic membranes predominate over cell bodies) membranes are also rich in glycolipids. They are integral part of the leaflet and the charged part lies in the external charged gel analogous to the mucins showed in Fig. 3. These lipids can carry up to seven charged groups of sialic acid and they are estimated to be 15% of the total lipid of central gray matter (for literature see Fernandes de Lima et al., 1997b). Rahmann et al. (1992) proposed that the charged heads would function as attractors to screening calcium ions and therefore would contribute to excitability control within the neuropil. In short, the glycosides would diminish the calcium activity. This concept agreed with the results of our experiments using the observation of the IOS of retinal waves (Fernandes de Lima et al., 1997b): gangliosides were more effective in decreasing excitability than sialic acid, EGTA and phosphatidylserine. The effect was time and negative charges in headgroup dependent. This result could be predicted from Ichigi Tasaki membrane model and could not from pores and circuit equivalents models. Gangliosides slowed down retinal waves propagation velocity, decreased the peak amplitude of the IOS and accelerated its recovery.

The second optical component of the IOS of retinal waves coincides with accelerated glycolysis and lactate production by glia and thus is blocked by metabolic blockers. Its onset is gradual not abrupt, the kinetics measured in minutes and not in seconds. That both components are dominated by glia membrane events is an interpretation that the chick retina gives to us for free. In this avascular retina, the pecten is continuous with the sclera and in front of it lies the optical papilla, the place of convergence and output of the axons that make the optic nerve. There is no cell bodies, no synapses, only glia processes (fine tubes) and axons. It is long known that axons contribute little to the macroscopic hydroionic changes of spreading depression waves (here assumed as excitation waves in excitable media). The IOS of retinal waves does not change at the optical papilla. Therefore, the signal we see - the macroscopic IOS- of retinal spreading depression is dominated by glia membrane events.

The consensus about the second component of the spreading depression waves IOS is that it is tightly coupled to acceleration of metabolism and lactate production by glia that secretes lactate in the extracellular gel. Observed in cortex, cerebellum and retinas

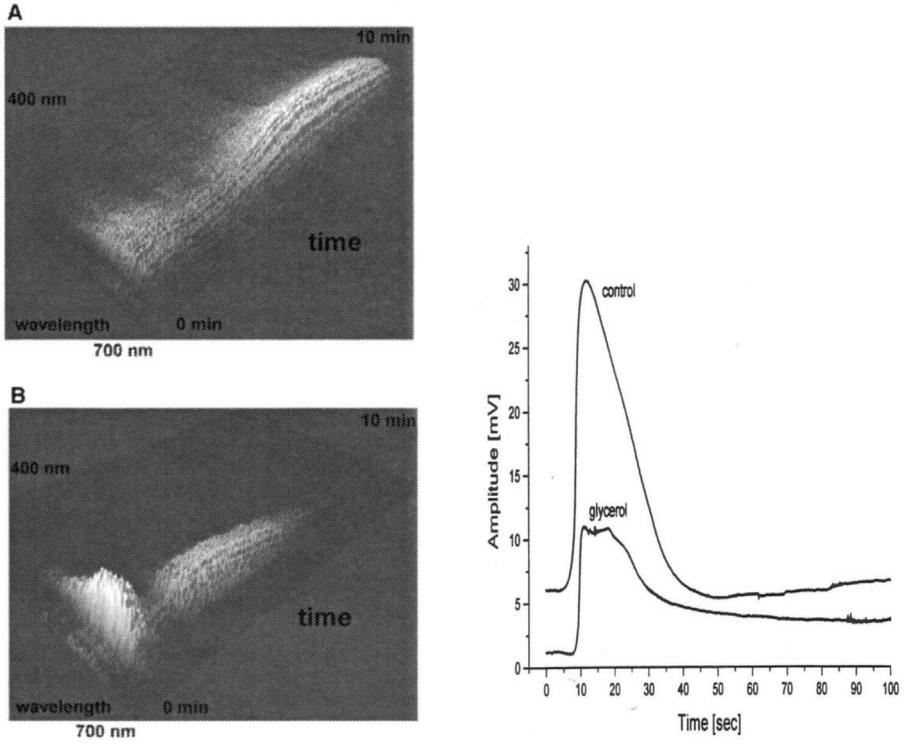

Fig. 8. Bellow control (B) time/frequency spectrum recorded in similar conditions as in the previous figure and above (A): the effect of 5% volume addition of glycerol. Note the abrupt onset of the first optical peak and the gradual one of the second optical peak. Glycerol depresses the first peak of scattered light and delays the onset of the second peak also flattening it. It does not change the onset of the potential drop but depresses its amplitude and increases it duration in a similar way it does to the first optical peak of the spectrum. On the side the temporal evolution of the potential drop of a control wave and a wave obtained in the presence of glycerol 5% added to the maintenance solution. The amplitude is depressed and the first peak duration increased. Modified from Fernandes De Lima et al., 2001 and Peixoto et al., 2001).

(Chesler an Kraig 1987, 1989; Casper et al., 1997).

In Fig. 9, we show the result of one experiment in which the eyecup preparation was exposed for 35 minutes to a solution with 29 μM SNARF-AM calcein. Then perfusion was reinstated and maintained for the time it took to change the total volume 3 times over. Then waves elicited mechanically. The figure shows the IOS recorded at 500 nm (illuminating light) and at above 580 nm (red band pass filter in front of the camera). Both images recorded simultaneously as a single frame so that the same mathematical filters applied to both sides during imaging processing (lowpass spatial filter). Two consecutive frames 10 seconds apart were subtracted from each other so that the open circle image shows the 10 seconds propagation of the wavefront, the calculated propagation velocity was 2.7 mm/min. The open circle shows the light scatter at 500 nm and this wavelength overlaps the fluorescence emitted by the dye with intracellular alkalization, most of what we see is probably light scatter of the illuminating light. On the side, the optical signal at orange/red. Here we see first a reduced signal to noise ratio, second dark pockets that could be due to the alkaline shift and the bright ones the early red scatter that we propose is dissipation of interfacial water energy. Note that in the region where the "classical" IOS is not present, red scatter is there. One possibility first described by Williams (1970) and endorsed by Tasaki (2008) is a process of saponification of lipids by potassium in patches of the bilayer; these could trigger a phase transition within the bilayer as part of the optical changes. This further structural change would follow the "volume phase transitions" of the polyanions in the external leaflet and could be the mechanism behind the blue/green light scatter, part of the classical IOS of retinal waves. As already pointed out earlier, dV/dt and d[K]/dt coincide in time as well as the peak of the early red scatter and intracellular calcium activity increase. Note that propagation is not continuous in space, but pockets of tissue differ in their state. In other words, an inserted electrode would show different results depending of its position, making easier to understand the results of glycerol shown in Fig. 8, observed in 50% of measurements.

In summary, the early red and infrared scatter is the macroscopic observable of the burst of energy dissipation at wave onset or propagating wavefront. We propose this energy coming from dissociated interfacial water becoming liquid water. This mechanism is in accord with the alkaline shift and heat dissipation measured at both action potentials and retinal waves (Tasaki and Byrne, 1991 a and b).

In Fig. 10 we show an image extracted by a B-Z gel that the authors called "Artificial Retina"; the physical chemical version of how retinas work, an alternative to the well-known silica version based on lateral inhibition of the neuronal circuit. The important statement in our opinion is the fact that the image was not static but **it flowed**. If we prepared an eyecup with the posterior chamber glued to a Petry dish, we could flash one image from above at slow frequency (1Hz) for 3 minutes and at the "off" of the pattern, begin to record with an infrared camera at a high rate like 300 Hz. If we are correct, a flowing image will appear and grow before disappearing. The recording time should be 2–10 seconds. We will see energy dissipation in the form of mechanical coherent flow and red and infrared light emission. A feasible experiment that can falsify our hypothesis.

Artificial lipid bilayers and biological membranes, their potentials and phase transitions- Artificial lipid bilayers are

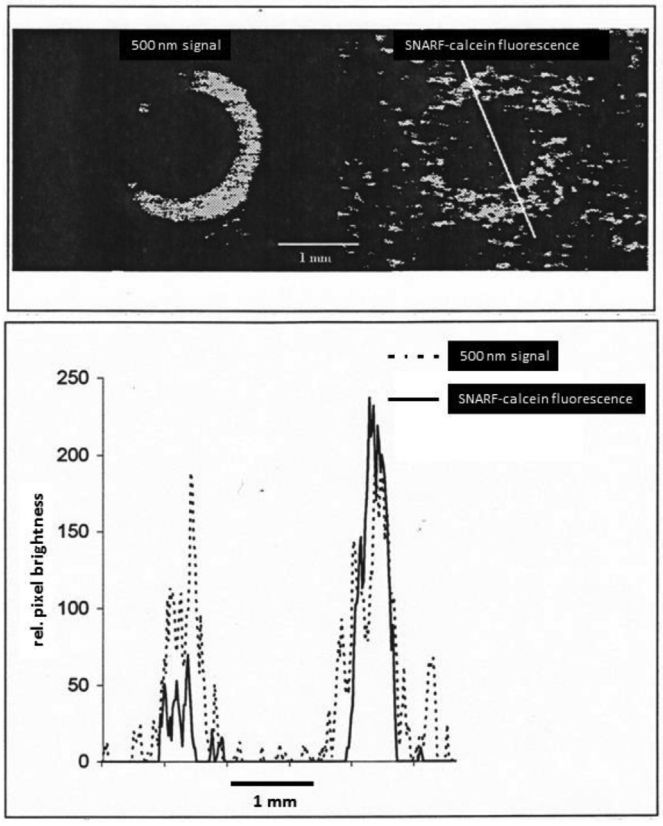

Fig. 9. Simultaneously recording at illuminating light (500nm filter- 480–520 bandwidth) and passband filter above 580 nm (−3 dB). Experiment with intracellular pH dye SNARF-AM [4]. Below optical spatial profile of the line shown above. Open circle shows the classical short wave light scatter typical of retinal spreading depression wave. Note that at the place where the classical IOS is missing, there is a orange/red scatter compatible both with the early red scatter and an intracellular acidic shift. The x axis of the profile is in pixels and 90 pixels ~ = 1mm. The traced line is the profile in orange/red and the full line the blue/green. Modified from V.M. Fernandes de Lima et al., The retinal spreading depression. ISBN-3-8265-4685-7, Shaker Verlag Aachen, 1999).

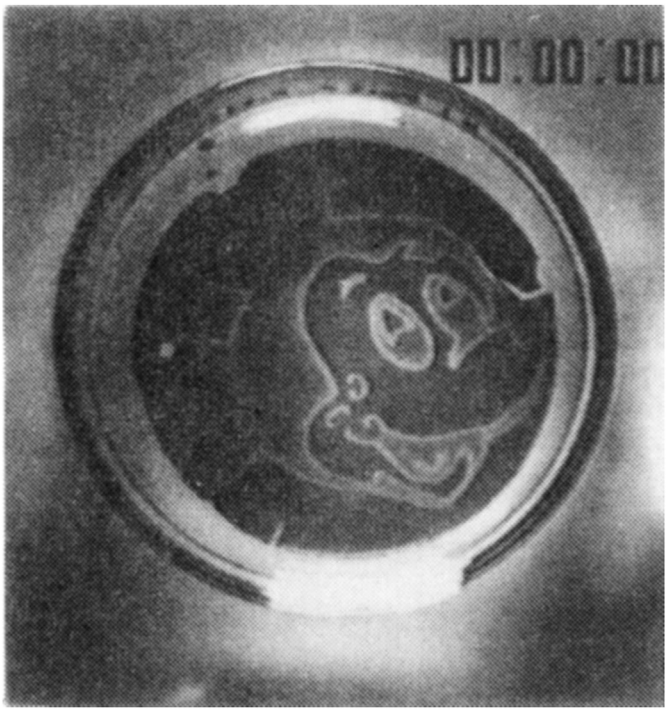

Fig. 10. Image extracted by a very thin (0.5 mm) light sensitive B-Z gel. The image grew or flowed. Modified from (Shikirin et al., 1992).

accepted as reductionist model of biological membranes and reconstituted proteins extracted from biological membranes can be incorporated in such bilayers (Hanke and Schlue, 1993). Assuming also that the properties of the reconstituted protein in artificial bilayer are the same as the protein interaction in the natural environment. Lipid bilayers are usually symmetrical and made with only a single phospholipid. Natural bilayers are asymmetric in lipid composition and glycolipids are important part of excitable membranes (Rahman et al., 1992). All intrinsic (meaning they are deeply set within the bilayer) proteins labelled receptors, channels and transporters are glycoproteins; mucins, schematized in Fig. 2 are an example of intrinsic glycoproteins. The functional channel or transporter is not one protein but a consortium of several sub-units that cooperate in order be a functional unit. Note also that there is no bilayer without water, therefore an artificial bilayer is interfacial water + lipid bilayer, the interfacial water in artificial bilayers named "unstirred layer". Fig. 3 shows that if water and lipids interact one gets a lipid monolayer at the interface.

In 1980 Boheim (Boheim et al., 1980) published a report on experiments that showed that at the critical temperature from jellified to liquid lipids, currents were measured that were undistinguished from the currents obtained with incorporated 'pores" (gramicidin). Carriers (Valinomycin) changed the behavior of the bilayer such that its response was qualitatively diverse from the pure lipid bilayer without changing the critical temperature. By contrast, gramicidin A and alamethicin, induced a new maximum of activity bellow the critical temperature. Although stark reductionist, when compared to biological membranes, the bilayer is not a simple, but it is a complex system and with incorporated peptides, its complexity increases. Their behavior during experiments are as hard to interpret as are isolated axons.

The 1980 lipid phase transition experiments pointed to a limitation of the methodology: individual current events at critical temperature, cannot be distinguished from supposed channel opening when peptides are incorporated in artificial bilayers. This often-forgotten interpretative complication was brought to the point in a paper by Thomas Heimburg laboratory (Laub et al., 2012). The authors compared the behavior of bilayers with biological membranes that expressed TRP (temperature receptors) channels. Again, one could not distinguishes between lipid phase transitions and channels responses. However, his paper had little effect on the faith of dozens of researchers that keep finding that TRPs also respond to pressure without even thinking in membrane physical chemical terms. Another contribution of Thomas Heimburg is his work with anesthetics (Heimburg, 2014), interpreting most of their effects through physical changes in the lipids bilayers.

It is also a puzzle that molecular biologists find that a change in a single amino acid modify qualitatively a protein function, but the fact that intrinsic membrane proteins in order to be incorporated are first "purified" i.e. they are separated from their neighbors lipids and their carbohydrates (glycosilation) are also lost. This detail is glossed over and the behavior found in the incorporation within artificial bilayers taken as the only one possible for the protein complex.

Another aspect little discussed is the numerous causal links attributed to calcium ionic activity and is absence in the model to explain rest potentials of excitable biological membranes. What happens to GHK equation when calcium is added is displayed below:

$$E = \ln\frac{RT}{F}\cdot\frac{p_k[K^+]_o + p_{Na}[Na^+]_o + p_{ca}\sqrt{[Ca^{2+}]_o} + p_{Cl}[Cl^-]_i}{p_k[K^+]_i + p_{Na}[Na^+]_i + p_{ca}[Ca^{2+}]_i + p_{Cl}[Cl^-]_o};$$

Well it becomes non-linear and thus analytical solutions are no more possible. Bellow we show a textbook figure common in sections about membrane potentials (for example Mountcastle Medical Physiology 13th edition). Note that Nernst equilibrium potential for potassium applies to a region far from the physiological range (see Fig. 11).

An account of how Nernst potential came to be adopted as the explanation for the rest potential in axons and neuronal cell bodies, was made by Gilbert Ling (2007). He also showed that blocking metabolism did not impede the usual distribution of sodium and potassium between extra and intracellular compartments. Later on, these concepts were incorporated into the Hodgkin-Huxley action potential model with the electrical circuit equivalent of the membrane. A detail in this conceptual arrangement is that the association of proteins called sodium and potassium channels receive the electrical equivalence of passive resistors allowing ions to pass descending electrochemical gradients. It happens that sodium and potassium differ greatly in their interaction with water: sodium is more electrodense and its solvated cover of immobilized water makes the ion larger than the potassium ion that interacts less and has less water immobile around it. At the channel opening, a miracle happens and energy comes from the Universe to make the ion sodium loose all its water, becoming smaller than the potassium ion that cannot go through the sodium channel by being too large. Another detail is that in the equivalent circuit capacitance is a constant, and the optical signals of excitation waves strongly support structural changes as well as the mechanical concomitants of action potentials and retinal waves.

In this paper we discussed the integrative power of Aharon Katchalsky, Eric Neumann and Ichiji Tasaki approach to synapses and action potentials. First the concepts derived from physical chemistry of colloids can be applied to action potentials, spreading depression waves and one heart beat as well as the depolarization that precedes CNS lesions following trauma or anoxia. Neumann and Katchalsky (1971) proposed: …" "Controlled changes in the environment of metastable macromols or subcellular macromol. organizations such as membranes by high elec. fields or by ion

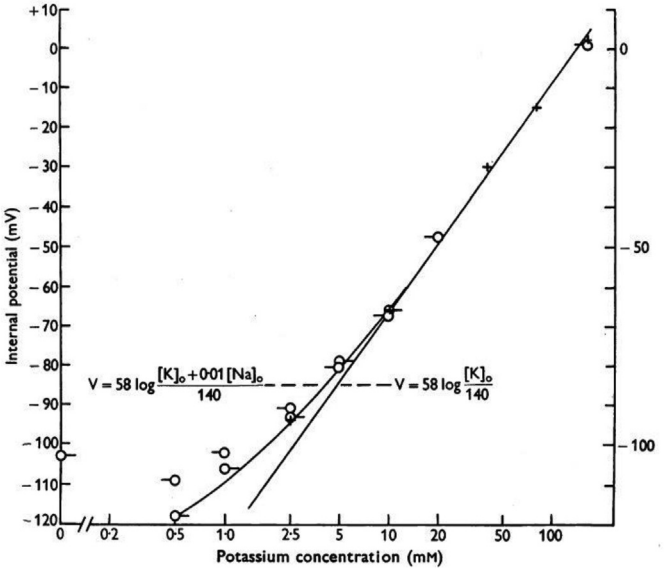

Fig. 11. Textbook depiction of membrane potential. Note that the abscissa is in concentration units, valid only in the case of diluted solutions of ions whereas around charged gels ion activities can vary without overall concentration changes. Note also that Nernst equilibrium potential for potassium only is found in the region far from the physiological range. To explain the deviation from Nernst, GHK proposed linear sum of other ions contributions. Calcium is ignored.

gradients can induce conformational changes which could serve as reproducible imprints of a memory nature." They made clear the wide implication of this view of CNS.

Second, we aimed at presenting the contribution to the field of the observations made in isolated chick retinas:

a) the fact that macroglial membrane state appears to be the dominant factor in the macroscopic IOS; b) with the creation of optical profiles back in 1962 by Hiss Martins-Ferreira and Gustavo Oliveira e Castro, one can clearly see that the membrane events at onset of spreading depression waves (excitation waves) and excitotoxic responses are identical. They differ in the temporal kinetics of the IOS, in the mode of spreading and in their outcome. Reversible in the waves and with irreversible sequels in the excitotoxic response of any origin (Fernandes De Lima and Hanke, 2012); c) the measurement of the spectrum of the scattered light and the discovery of the early component in the red/near infrared part of it. This finding opens the possibility of new experiments aimed at measuring the release of energy at wavefronts beyond the measurements of Tasaki and Byrne (1982, 1999).

In medicine, the integration principle is a rarity, for example, Mutch and Hansen (1984) published a series of experiments aimed at solving once for all the origin of the pH changes observed in spreading depression waves and cerebral ischemia. They could not distinguish the onset events; nevertheless, they did not assume they could be identical, and the temptation is to speculate as distinct events was irresistible. They proposed an "anion gap" or missing negative charges that could not be explained with classical membrane theories (i.e. assuming diluted solutions across a constant capacity semi permeable membrane model of Nernst). Spreading depression waves had at onset a membrane breakdown (whatever that means) and selectivity was lost. However, these assumptions did not agree with their experiments, because they attributed the alkalization to bicarbonate synthesis, a metabolic response. No matter, the model went in convoluted discussion about anion gap. In 1993 we heard the same missing anion account in a presentation by Charles Nicholson in a meeting. One of the authors would follow his talk with the good news that we just had done patch-clamp experiments in intact retinas (Hanke et al., 1993) and could measure channel activity in both glia and ganglion cells bodies in response to light, and when a wave invaded the region around the electrode. It turned out that again, the light response and wave response at the molecular level (today people would call it nanoscale) looked very much the same. In other words, there was no sign of a membrane "breakdown". We also looked for anion currents but did not find them; therefore, assumptions were wrong. The good news did not look so good to Charles Nicholson (1993) who became very angry at being called wrong in a meeting. Later, he wrote a preface in the book that summarized the experimental results presented at that meeting. We quote…"Everything is in flux during spreading depression and because of that, cause and effect seem no longer to apply …. After all these years this time and all these able minds, we do not know what causes spreading depression, how it propagates or why it ceases." … It is high time to change that. Ichiji Tasaki membrane model can explain propagation of excitation waves, Thomas Heimburg and Mathias Schneider are right when they call the attention of researchers to lipid bilayer importance in membrane behavior, however the proteins are there for a reason, and the membrane asymmetry must play a role as well.

Our experiments with liquid deuterium in B-Z systems and retinas made it clear the role of dissociated water energy in both systems. This energy can explain the burst of energy dissipation in excitation waves at one, two and three dimensions. Interfacial water goes from an ordered spatial structure with coherent motion to liquid water higher entropy and back during absolute refractoriness. This energy burst is tightly coupled to "volume phase transitions "in the electrolytes intrinsic to the bilayer and phase transitions at the bilayer itself can explain the optical signals observed.

In excitable media between state A (for example, quiescence) and B (recovery or acute cell lysis), there will be innumerable possible pathways, for each experimental context, a different probability distribution for these pathways. This concept is not popular in medical research no matter its integrative power. For example, the isolated retina experiments showed that independent of the means to elicit it, excitotoxic responses had the same onset. The onset was also identical to spreading depression waves. Furthermore, the experiments showed that lack of energy substrate did not explain the evolution to acute cell lysis or tissue death without it (apoptosis)- in this retina, all the metabolism depends on glycolysis by glia and in the bath there was plenty of glucose. Nevertheless, the tissue response to ouabain or glutamate was cell death. It should be noted that the macroglia in the chicken retina has few mitochondria positioned close to the ganglion cell layer. In the synaptic layers, the macroglia has no mitochondria.

2. Notes

Note 1- The uploaded poster is accessible in Research Gate in Vera Maura Fernandes de Lima page under contributions. In there the results show parallel global effect in both systems, as well as the fact that while the shape of individual cycles in B-Z bulk solutions reaction system was not affected, suggesting the chemical kinetics was not affected, the shape of the optical profiles of retinal spreading depression waves was deeply affected by the change in the solvent.

Note 2- Elmar C. Fuchs has uploaded free access videos shown in the paper in the Journal of Visual Experiments (JoVE), all the features of the transition from quiescence to steady state far from equilibrium are shown in the water bridges. These features are common to all dissipative structures; not only water but also other polar liquids form bridges. We quote … "A number of common solvents can form such bridges as well as low conductivity solutions and colloidal suspensions." … Or one can watch these free access videos in YouTube: https://www.youtube.com/watch?v=FhBn1ozht-E; https://www.youtube.com/watch?v=B27P12B5yZs; https://www.youtube.com/watch?v=N1At3Gcd-No&t=297s.

Note 3- the following videos were also uploaded in one of the authors home page in Research Gate: DOI 10.13140/RG.2.2.27416.29447 Video shows what light touch is to get a retinal wave with mechanical stimulation (2 seconds video); DOI:10.13140/RG.2.2.22383.12960. This video shows the central retina. The circular wave invades the optical papilla, the region in front of the pecten, the dark structure seen in the movie. This video and the previous one are played at the real time or acquisition time. The X axis length is about 2.7 mm in both videos. The video named STACK-5 is played at 5 times slower frame rate that the acquisition time, the signal was acquired in 12 bits black and White and displayed in 12 bits false colour display with from black, deep blued to yellow and White. Black 0 brightness white 256 brightness. It is additional material to the paper showing the non-linear coupling of optical and electrical events in retinal waves [45]. The width of the innerplexiform layer is 100 μm. DOI:10.13140/RG.2.2.13601.51045. This video was uploaded to show how the simultaneous recording of extracellular potential drop and the Intrinsic optical signal in vicinity of the electrode tip was recorded together with the video frames of retinal propagating waves. The length of the X axis is 2.3 mm in this video. The two videos should be played with maximum resolution so one can see the shadow of the recording electrode

inserted in the innerplexiform layer.

Authors statement

Both authors, Wolfgang Hanke and Vera Maura Fernandes de Lima of the manuscript:

Have equally contributed to it in any part of the manuscript, including :

Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/ Writing - original draft; Writing - review & editing.

Both authors agree with this statement.

Acknowledgments

This paper is dedicated to the memory of Aristides Leão, Gustavo Oliveira e Castro and Hiss Martins-Ferreira. Three outstanding Brazilian neuroscientists.

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