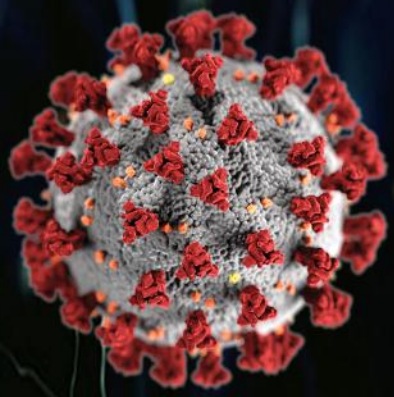
Computation and sentience in the brain with a parallel study on the importance of Human Serum Albumin (HSA).

Andrew S Johnson



DOTTORATO DI RICERCA IN BIOLOGIA

PhD Thesis

DATA INIZIO/FINE 2019-2022

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INDEX

1. *Acknowledgements.*

I would like to thank Professor William Winlow for his help and advice, Gianluca Polese, and my colleagues and friends in Napoli for their support. I would also like to thank my wife and family for their help in this scientific venture. In addition, I would like to thank all other scientists who have cited and followed my work.

2. *Aims and following discovered leads*

Science is the exploration of the unknown. Sometimes initial research into one subject follows its own trajectory into another, important events change lines of research. I investigated one specific problem in neuroscience, and this has led to a discovery in albumin function that might have very positive implications in many, if not all areas of medicine.

The initial aim of the research was to examine the relationship between plausible computation theory of the action potential and to follow logically to the areas of the brain and to examine perception and learning memory and thus sentience. All this depends upon the health and the age of cells and when, incidental to the thesis, discoveries were made (Cardiac Action potential Pulse, Pre-eclampsia) these were published, and I include a full list and addendum.

Conventional computers work by timed clock gating-nodes. However, the brain is unsynchronised and therefore Neural messaging is based upon the action potential being able to transmit messages by frequency. This we demonstrated both from legacy unpublished experiments from Prof Winlow and by describing the necessary computational elements. I also investigated many forms of computation and compared the deconstructed elements to form a separate theory of phase computation based upon the retinal network. Work in the study of computation in the brain has nevertheless continued with papers published every year with many citations for each paper from other investigators. The research for this thesis has examined computation in the brain in the context of both the well and unwell body.

Initially a thesis on computation in the brain, with the onset of COVID-19 a parallel investigation was commenced into nutrition and its effects on computation in relation to the nervous effects of the virus. The investigative work for this has been extensive across many subjects and has covered most systemic organs and compared vulnerabilities to function in context with the components of the blood (whole, plasma, interstitial fluid, lymph), initially to modulate the cells of the brain behind the blood brain barrier (BBB).

During the early part of 2020 during lockdown, I shifted my attention to COVID-19. I looked first for common factors among those struck down with the disease and noted that those most affected suffered from sepsis-like symptoms or had vulnerabilities that gave a greater risk of sepsis. My investigation of sepsis revealed that sepsis is caused by a disruption of the endothelial glycocalyx. Because sepsis symptoms are systemic, I looked for a common factor in the systemic medium (blood, plasma, interstitial fluid, and lymph) that occurred during illness (in this case COVID-19) and

noted that Human serum Albumin (HSA) levels decrease over 50 years of age. I found many other common factors and was able to elucidate from this that albumin binding deficiency (ABD) is involved with some or all vulnerabilities to COVID-19. I researched fluid therapy and discovered that also HSA had been used its effectiveness was questioned. I realised that present fluid therapy techniques were inadequate to raise HSA levels as infusion was through the periphery which led to colloidal pressure and nutrient imbalance in structures leading to degradation. On investigating I realised that problems could be avoided by infusing direct to the liver through the hepatic portal vein (HPV). I conclude that raising HSA through the liver will raise the colloidal pressure and the whole blood volume (WBV) at the same time as increasing essential nutrients.

3. Main Discoveries

Neuroscience

- Transmission of nerve impulses for computation is by a frequency based combined mechanical and Hodgkin Huxley action potential which we have called Action Potential Pulse (APPULSE).
- The active computational elements of the APPULSE are the resting, threshold and refractory periods that have the ability to annihilate other converging APPULSEs to produce Phase Ternary Computation (PTC).
- PTC involves quantum phase computation.
- The retina computes by changing action potential frequency in response to light and I was able to deconstruct the retinal circuits and evaluate the frequency results to show that both the APPULSE and PTC combine in the neural circuits of the retina and by extension to the rest of the brain.
- Perception and sentience are maintained by PTC.

COVID-19

- Whole blood volume (WBV) maintains the health of all cells. Human Serum Albumin (HSA) is the main determinant of WBV and maintains levels by the HSA lymphatic pump (HSALP).
- Every other component in the blood of a healthy individual is controlled incidentally by the central process of albumin level: blood pressure, colloidal pressure, nutrients, waste removal.
- Albumin binding deficiency causes sepsis.
- In COVID-19 excess binding ligands from COVID-19 cause albumin binding deficit (ABD) leading to further damage. We propose this is the mechanism for the serious vulnerabilities to COVID-19 and sepsis.
- We propose that raising HSA levels will alleviate many symptoms and provide evidence that this can only be done by infusion of HSA direct to the liver.

4. Introduction and Overview

4.1 Neuroscience research was performed by an integrative investigation of computational methods, comparing what might work within the known structure and physiology of the human brain and eliminating incompatible methods. I discovered that the most likely method of computation is phase

ternary computation, which I describe in detail. I then confirmed my theory by redescribing available evidence from other experimental researchers of the eye and ears. In the retina we discovered that frequencies of input and output can be described by phase ternary computation (PTC), when considering the connectivity of the action potential pulse (APPulse). I described mathematics that exactly predicted the results recorded, demonstrating the principle of phase ternary computation in the eye. The nature of the action potential, whether it propagates by the Hodgkin Huxley mechanism alone or by soliton has long been contentious. In this research we demonstrate that the APPulse combines and synthesises two other theories, the Hodgkin-Huxley theory of action potential propagation and the soliton theory. I have described a quantum computational method (phase ternary computation) that corresponds in context. In addition, previously unpublished laboratory work by Prof Winlow from the 1980's was used to explain the latency aspects of the APPulse and provide further evidence for the thesis. Further investigation is proceeding with an explanation of active memory and the visual cortex which we hope will be published imminently.

4.2 COVID-19 research. Publishing delays at the start of COVID-19 partly changed the focus of my thesis to illnesses of the brain and to consider the basis of cellular health as being dependent in its surrounding medium. COVID-19 is a systemic disease, that when serious, affects the whole body in a systemic manner. We therefore searched for common factor(s) that could explain the systemic degradation and systemic spread. Our research led us to the systemic plasma protein human serum albumin (HSA), which we have now theorised is the active determinant of whole-body fluid levels, blood pressure, cellular nutrients, and waste removal.

My research has been on integrative biology and physiology and has not been confined to any one subject. I have documented many clinical implications/diseases from many different subjects that might be alleviated according to our research. On the systemic level the ability to change whole body fluid levels has clinical implications in many areas of medicine because it defines the precise concentrations of 0_2, CO_2 , nutrients, pressure, and other material in the blood. Successfully raising HSA may be a solution to the problem of ensuring adequate nutrients to cells during infection or illnesses of the brain which were examined in relation to computation in the brain (above). Added evidence for this comes from studies (COVID-19 and Sepsis) where statistics of vulnerable individuals can be shown to suffer HSA binding deficiency and age studies showing degeneration of HSA in the over 50's. Using available data where HSA has been applied, we have demonstrated that peripheral infusion leads to degradation of the nutrient systems and destabilisation of the colloidal pressure normally governing interstitial spaces. HSA is controlled in homeostasis by a pressure feedback loop centred on the liver where it is manufactured. A successful addition of HSA causes a chain of events first enlargement of body fluids secondly binding of albumin to nutrients in the liver and restoration of Hb levels. I proposed therefore that infusion be given to the liver. Infusion of the liver has the benefit of HSA leaving having been charged with nutrients thus maintaining both colloidal pressure and nutrients in situ. There has been understandably interest in the newly created subject of HSA liver infusion, and we are in contact others who might help us take the technique to trial. There are many clinical benefits of being able to control body fluid composition. The conclusion is that albumin (HSA) level, distributed in the organs may be the rate limiting factor for stability and recovery in many illnesses including COVID-19.

5. NEUROSCIENCE THE STORY

This was a search for the most plausible method of computation that could exist within a neural network.

- I investigated the nature of the action potential in this paper and noted the possibility that colliding action potentials annul and that this was due to their refractory periods. My investigation showed that the action potential is propagated by mechanical force from an associated soliton pulse which passes along the neuron concurrently with the action potential. We demonstrated in this paper how mechanical opening voltage-gated sodium ion channels caused the HH action potential but could also account for the speed along a neuron and computation – which HH cannot.
- I showed that the action potential peak cannot be used for computation and that the moment of computation depends upon the threshold instant.

In neuroscience our original hypothesis of the APPulse (a soliton pulse synchronised by the energy of the Hodgkin-Huxley ion exchanges) is now being taught – or at least mentioned as an alternative to the Hodgkin Huxley action potential in many universities. We have been cited many times and statistics continue to show support. Continued reviewed interest in this subject has transpired since and is continuing in 2022-2023

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5.1 Johnson AS and Winlow W (2018) ***The Soliton and the Action Potential – Primary Elements Underlying Sentience.*** Front. Physiol. 9:779. Doi: 10.3389/fphys.2018.00779

After this was published, we had extended correspondence from dedicated Hodgkin Huxley fans. They suggested that we were wrong, we wrote back to the editor destroying each and every of their arguments. The discussion has now migrated from Physiology News to ResearchGate. I include a citation here as it is a side-line to the thesis, but the discussion is nevertheless of interest.

United Kingdom 1 – Italy 0 => Science loses Delalande B, Tamagawa H, Matveev V DOI: 10.13140/RG.2.2.16096.71682

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REVIEW
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The Soliton and the Action Potential – Primary Elements Underlying Sentience

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At present the neurological basis of sentience is poorly understood and this problem is exacerbated by only a partial knowledge of how one of the primary elements of sentience, the action potential, actually works. This has consequences for our understanding of how communication within the brain and in artificial brain neural networks (BNNs). Reverse engineering models of brain activity assume processing works like a conventional binary computer and neglects speed of cognition, latencies, error in nerve conduction and the true dynamic structure of neural networks in the brain. Any model of nerve conduction that claims inspiration from nature must include these prerequisite parameters, but current western computer modeling of artificial BNNs assumes that the action potential is binary and binary mathematics has been assumed by force of popular acceptance to mediate computation in the brain. Here we present evidence that the action potential is a temporal compound ternary structure, described as the computational action potential (CAP). The CAP contains the refractory period, an analog third phase capable of phase-ternary computation via colliding action potentials. This would best fit a realistic BNN and provides a plausible mechanism to explain transmission, in preference to Cable Theory. The action potential pulse (APPulse), is made up of the action potential combined with a coupled synchronized soliton pressure pulse in the cell membrane. We describe a model of an ion channel in a membrane where a soliton deforms the channel sufficiently to destroy the electrostatic insulation thereby instigating a mechanical contraction across the membrane by electrostatic forces. Such a contraction has the effect of redistributing the force lengthways thereby increasing the volume of the ion channel in the membrane. Na ions, once attracted to the interior, balance the forces and the channel reforms to its original shape. A refractory period then occurs until the Na ions diffuse from the adjacent interior space. Finally, a computational model of the action potential (the CAP) is proposed with single action potentials significantly including the refractory period as a computational element capable of computation between colliding action potentials.

Keywords: sentience, action potentials, soliton, phase ternary computation, brain neural networks

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INTRODUCTION

Sentience may be thought of as the highest ability to perceive events in the context of previous or future events, resulting in conscious non-reflex behavioral modification(s) and is dependent on self-awareness. Sentience must encompass elements of both time and complexity and is dependent upon individual experiences. The generation of sentience and other behavior must depend upon the brain's ability at the level of neurons to compute nerve impulses according to timing defined by the biological processes present.

To understand how we compute sentience we must first understand how action potentials compute in temporal space within the brain neural network (BNN). These computational mechanisms are traditionally described by the action potential (Hodgkin and Huxley, 1952). The Hodgkin Huxley equation describes the potential across the membrane of a neuron in terms of ion exchange changing over a period of time. The timing of the charging and thus the speed of propagation is defined by Cable Theory. It was assumed in 1952 that excitable membranes contained sufficient ion channels close enough together that the spread of charge from one channel could affect another. We now know this is not the case and an alternative method of propagation must be taking place to account for the speed of propagation. A problem is the lack of knowledge about the fundamental and computational mechanisms that underlie the generation and propagation of action potentials in single neurons and neuronal networks. A mechanical pulse known as a soliton always travels with the action potential at the same speed which has been considered ancillary, in this paper we show that it is this pulse that defines the speed and thus the computational mechanisms that form the basis of behavior.

Neurons are diverse and have many shapes, sizes and functions (Bullock et al., 1977). They may have evolved from secretory cells in the early metazoa. We can envisage that as animal size increased the action potential evolved to control secretions at a distance (Grundfest, 1959; Winlow, 1990) although many local circuit neurons in both vertebrates and invertebrates do not conduct nerve impulses (Dowling, 1975, 1992, chapter 13; Shepherd, 1975, 1988, chapter 10; Roberts and Bush, 1981). However, the discovery of the nature of the action potential, which is used to signal over distance, was critical to the development of modern neurophysiology. Unfortunately it has been modeled as a binary event in computational brain networks (Johnson and Winlow, 2017b). We believe this assumption to have been unnecessary and to be the wrong premise for computation both within nervous systems and in the development of artificial intelligence (AI). Furthermore, the advantages of ternary computing over binary computation are that it requires less hardware and contains more information in a shorter code. Phase ternary computing results from phase addition of a ternary pulse. Here we discuss evidence that the action potential is a temporal compound ternary structure, described as the computational action potential (CAP).

The CAP contains the refractory period, an analog third phase capable of phase-ternary computation via colliding action potentials. This would best fit a realistic BNN and provides a plausible mechanism to explain transmission, in preference to Cable Theory. The action potential pulse (APPulse), is made up of the action potential combined with a coupled synchronized soliton pressure pulse in the cell membrane. We describe a model of an ion channel in a membrane where a soliton deforms the channel sufficiently to destroy the electrostatic insulation thereby instigating a mechanical contraction across the membrane by electrostatic forces. Such a contraction has the effect of redistributing the force lengthways thereby increasing the volume of the ion channel in the membrane. Na ions, once attracted to the interior, balance the forces and the channel reforms to its original shape. A refractory period then occurs until the Na ions diffuse from the adjacent interior space.

Finally, a computational model of the action potential (the CAP) is proposed with single action potentials significantly including the refractory period as a computational element and capable of computation between colliding action potentials.

MODELING THE ACTION POTENTIAL

A Lesson From Cephalopods

It is appropriate to this report that the physiology of action potentials was first modeled using the giant axon of the squid, *Loligo forbesi* (Hodgkin and Huxley, 1952). This model predicted the ionic currents crossing cell membranes to create a potential difference and changing over time due to the modulation of currents. However, one of the major problems in AI is how to code accurately for the action potential. Action potentials are critical to the operation of the brain and computation and timing of the action potential is important in considering any possible computational requirements. Thus, the mechanisms that define the speed of the action potential and its temporal accuracy will directly affect the methods of reliable computation available to the neural network. Thus changes in accuracy of action potential timing would make any form of computation unreliable.

The action potential can be divided into three computational phases, resting, threshold and refractory, the specific details of which are discussed elsewhere (Johnson and Winlow, 2017a). The first two phases may be modeled digitally, while the refractory phase is an analog event. Thus the action potential can be considered to be a phase ternary event. Phase ternary computation is an unexplored field in computation.

Action potentials travel at a speed commensurate with the membrane dynamics of the axon and have been shown to be accurate to at least 1 millisecond over its length in small neurons (Diesmann et al., 1999). The transmission dynamic of any axon or part of an axon may be different depending upon the membrane components such as the ion channel spacing (Hodgkin, 1975; Holden and Yoda, 1981; Hille, 1992) and the physical formation of the membrane.

The Macroscopic Point of View

Measurements of the action potential are taken from both sides of the membrane and measure the potential difference across a wide area reflecting the measurement of the H&H model (Hodgkin and Huxley, 1952). An action potential travels not

through the cytoplasm – where it is measured with intracellular microelectrodes – but is a product of the ion changes at the surface of the membrane. Small diameter axons (0.2 μm) have ion channels widely spread with low concentrations of ion channels (Holden and Yoda, 1981; Hille, 1992; Marban et al., 1998). All measured action potentials have been recorded at some distance from the membrane. As the action potential progresses, the micro-pipette measures current not from a point on the membrane, but from an area including multiple ion channels, and may not reflect the mechanisms of propagation from a single point. The same is true for the loose patch clamp method, where rather large (15–30 μm) (Marrero and Lemos, 2007) external patch electrodes are used and are unable to measure changes at a single point (Walz, 2007).

The Hodgkin Huxley Equation and Cable Theory

The H&H equation describes ionic flow across the membrane in mathematical terms over the period when the membrane reaches threshold until the end of the refractory period. The membrane can be considered polarized immediately the threshold takes effect, as after that point there is no return. After threshold the membrane is in the refractory state as no further action potential can be created. The action potential has a maximum speed of about 1 ms^{-1} in unmyelinated the axons (Waxman and Bennett, 1972). Thus, there is a 'leading edge' just before depolarization and as the action potential is self-propagating this leading edge must have the innate property of self-propagation. In effect it also instigates the refractory period once the depolarizing wave has passed. Patch clamping on single ion channels has demonstrated that the existence of threshold, spike and refractory period can be explained by modulation of the Na^+ channel alone (Holden and Yoda, 1981; Marban et al., 1998; Catterall, 2012). Thus the physical origins of the potential changes associated with the action potential can be directly attributed to the ion channel mechanisms, with the activity of the sodium channels defining its progression. This implies that the only element that is responsible for the live propagation of an action potential is whatever mechanism causes the threshold at the leading edge.

The Threshold Alone Is the Initiator of the Action Potential

It is also the rate limiter to the velocity of the action potential along the axon. The timing of the spike is therefore directly related only to the threshold. The threshold may be better defined temporally so that it is not a potential difference but a change over time in terms of action at the level of the membrane, i.e., the equilibrium point at which each subsequent ion channel opens. The predominantly voltage gated Na^+ channels (Yu and Catterall, 2003) open when positive charge approaches their s4 units. This all-or-none point is assumed to be the point of threshold. Conventionally, in the H&H model, threshold is assumed to be a direct result of Na ions arriving from a neighboring channel. For the APPulse structure **Figure 1**, the mechanical pulse opens the channel enough for the electrostatic insulation to break causing electrostatic attraction between the positive Na^+ ions passing through the s4 units and the negative parts of the ion gate structure. A mechanical contraction occurs transferring entropy (energy dispersal) directly to the soliton. Na^+ ions continue to be attracted to the negative intracellular space until the negative charge is balanced, at which point the gate is closed. The remaining positive ions adjacent to the negative regions of the ion channel prevent any further opening, and remain deactivated until the Na^+ charges diffuse into the intracellular space. This is explained in **Figure 2**, where a voltage–gated Na^+ channel is illustrated. The ion channel is therefore reactive both to mechanical and charge stimulus. Entropy from the soliton is only required to break the electrostatic-insulation of the ion channel for membrane contraction to occur, thus producing the next entropy charge for the soliton to proceed. The threshold is defined therefore as the time for the s4 units to fully open the channels. The rest of the action potential is only concerned with the refractory period and stabilization to resting potential. It is irrelevant to speed of transmission, although of course the refractory period can affect the frequency of transmission.

The Microscopic Point of View

At a microscopic level the mechanism of the threshold can be investigated knowing the properties of just the Na^+ channels and the membrane, for example using the patch clamp technique. The Na^+ channel has been isolated and its properties understood (Catterall, 2013; Shen et al., 2017). In addition, the speed and flow of an action potential is directly dependent upon the speed of activation of the threshold potential corresponding to opening of the Na^+ gates which may be achieved mechanically (see below), the resulting entropy causing a soliton. The H&H model is a proven advantageous means for the demonstration of ion transport and passage through a membrane over a large area, but because it cannot explain charge flow along the length of the membrane it must be allied with a mechanical model. However, it should be understood that because of the physical properties of Na^+ ions, threshold charge cannot flow from one ion channel to the next in the time available. There must therefore be another mechanism present and a 'soliton' mechanical pulse is known to be present. This is the force-from-lipid mechanical energy (Brohawn et al., 2014) that opens the Na^+ channels. This coupled APPulse fulfills the requirement for a continuing action potential where Cable Theory fails.

Cable Theory defines the potential arising from ion disparity across the membrane as a capacitance. Historically Cable Theory (Rall, 1962, 1995) comes from a direct analogy from capacitance theory and considers charge over the whole surface of an insulator (Poznanski, 2013). Thus, Cable Theory is a mathematical construct to predict observations on large areas of membrane, in which the depolarization of the membrane during the action potential is analogous to the discharge of a charged capacitor. Experimentally, the mathematics of cross current resistances appear to work correctly over a large part of the membrane in the macroscopic view and this is the basis for the H&H model. Cable Theory itself has recently been revisited to demonstrate that both neuronal morphology (Bestel et al., 2017) and the extracellular medium (Bédard and Destexhe, 2013, 2016) exert significant influences on neuronal cable properties as might

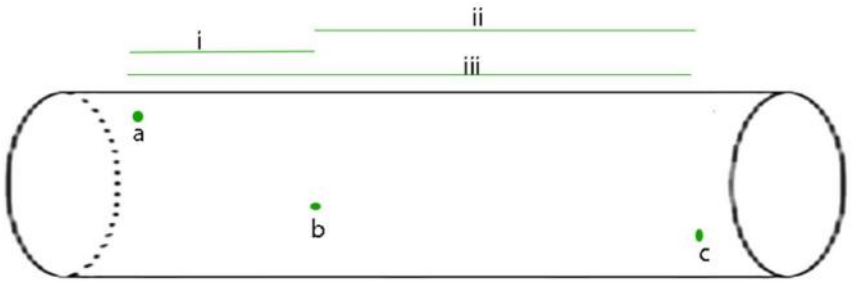

FIGURE 1 | The APPulse - an illustrative, uniform axon containing three widely spaced ion protein channels is shown. For the benefit of a clear description the axon has been standardized as follows: 1 the axon is uniform such that speed along the axon by the lipid pulse is constant; 2 - the protein channels (in green) are gates that reach threshold at a voltage of V and produce a digit of Entropy E; 3 - in this axon there are no lipid channels or other proteins except the three ion channel proteins, a, b, c. The diagram illustrates the events that take place for the APPulse to continue between ion channels. On depolarization at a, an action potential digit of entropy E is created. A Lipid pulse is subsequently created by Entropy E that continues along the axon. Entropy loss e from E causes a proportionate decrease in amplitude but the velocity of the soliton is constant where the membrane components are uniform. This causes the entropy to decrease by dissipation over distance d such that Ed(b) = E-e. This residual entropy Ed (b) is enough to mechanically distort the ion channel at b breaking the electrostatic insulation. Contraction of the ion channel ensues completing the mechanism. In this model the entropy of electrostatic forces produced by the ion channel at (a) must be sufficient to produce a lipid pulse of such entropy E to arrive at (b) with sufficient entropy to break the electrostatic insulation of (b) and repeat to c, where the process again repeats itself. In smaller diameter neurons dissipation will be greater due to entropy being a function of membrane area. i, ii, and iii represent different timings between separated ion channels along the membrane. From Johnson (2015) – reproduced under the Creative Commons License.

be expected. Such effects require considerable mathematical modifications to the original theory.

When H&H were conducting their experiments accurate depictions of the membrane, ion channels and specifically inter-channel distances were not available, but were assumed to be "separated by an infinitesimal distance" (Hodgkin, 1975). It was not until single patch clamping became available (Hille, 1992) that accurate measurements of channel density became known (Hamill et al., 1981; Goodman et al., 2005). At the time that H&H described longitudinal flow, the assumption was that there was a mechanism by which activation of an ion channel during threshold would activate adjacent ion channels. This was thought to produce a cumulative and on-going propagation – just as the charging electrons distribute evenly and almost instantaneously. However the mechanism of propagation between channels was not identified then and has still not been identified. Thus, the action potential takes place at the level of the membrane, which means that the resulting observation is due to all the combined ionic changes in a very wide portion of that membrane.

Mechanisms must exist in the membrane to produce an on-going pulse. Intra-Ion channel distances have been measured accurately although this distance is variable. Single patch clamping a membrane will usually only detect a single active ion channel (or possibly two) if the pipette is sized about 1.5 μm. As the membranes examined contain many different ion channels we can confidently say they are spaced at least 1.5 μm apart. Thus "Channels are not crowded" together (Hille, 1992, pp. 334–335) and the distance over which a single Na ion can affect another channel is limited by the time taken to travel through the ion channel and to the next channel. The ionic radius of Na^+ is about 116 pm (Shannon, 1976) indicating that a Na ion has to lie adjacent or in very close proximity to the Na^+ channel to activate it and the channels are ion specific. The time required for charge to spread from one ion channel to the next can be calculated from the ionic radii and the diffusion coefficient (Goodman et al., 2005). A conservative simple Speed-Time calculation suggests that the maximum speed that charged Na ions can travel between channels is less than 1/1000 of what is necessary for propagation (Johnson, 2015). Cable Theory only models the ion flows of the action potential under conditions of voltage clamp, but as yet there is no known mechanism for propagation of the action potential provided by Cable Theory. Thus, the H&H model very clearly demonstrates the electrically measured activity surrounding the underlying mechanism of propagation from one channel to the next, but not the mechanism itself.

IS THERE EVIDENCE FOR A MECHANICAL COMPONENT IN ACTION POTENTIAL PROPAGATION?

Two supposedly incompatible models for action potential propagation have been proposed and compared (Appali et al., 2012), the H&H model and soliton theory. However evidence at the level of the membrane structure suggests the two models are compatible and are synchronized; the H&H model at the macroscopic level and the soliton model at the microscopic level. In the H&H model of the membrane, for propagation to occur positive ions would have to behave as electrons but positive charges are a part of the atom and so physically a sodium atom must move for the charge to move. Without a mechanical component the H&H action potential cannot propagate. Recent evidence suggests that a "force-from-lipid" model (Brohawn et al., 2014) could transmit pulses into mechanosensitive ion channels in the absence of other cellular components and might also explain propagation through the membrane lipid. We envisage that it would take the form of a soliton known as an APPulse which would be the precursor of ion channel opening.

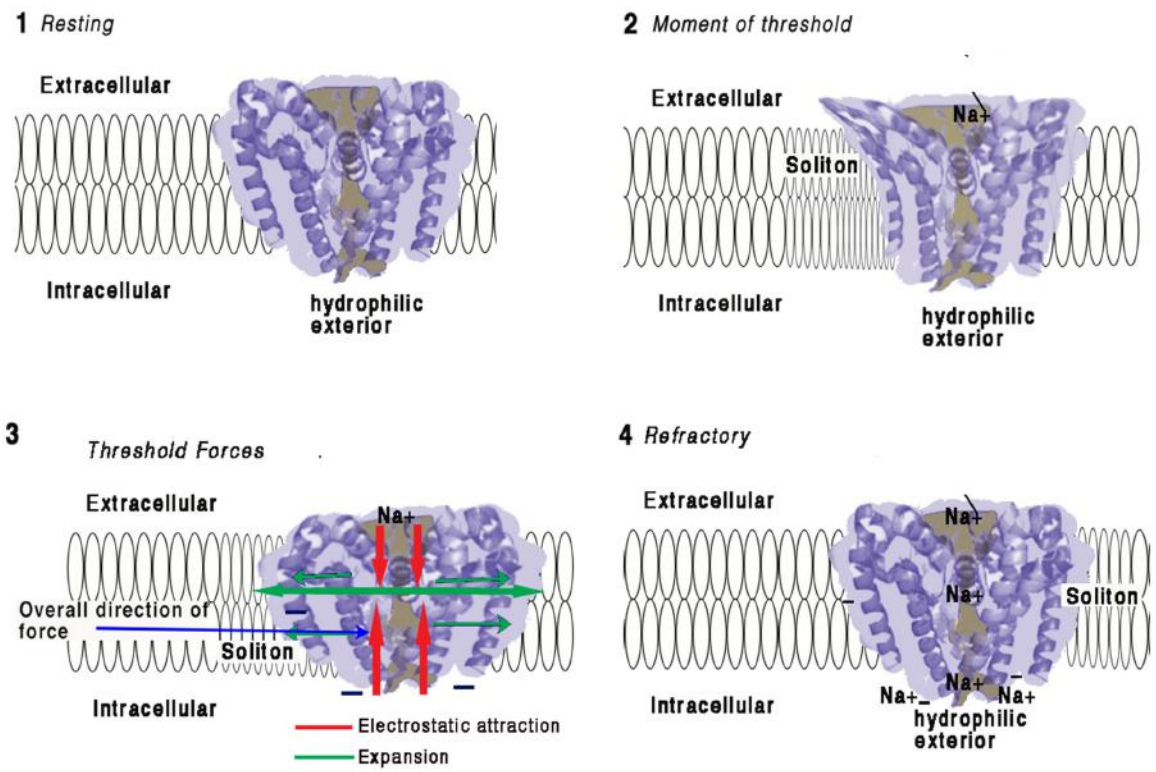

FIGURE 2 | Illustration of how force from lipid may act on a eukaryotic voltage-gated sodium ion channel from the American cockroach (adapted from McCusker et al., 2012 and Shen et al., 2017).

(1) Resting:

Ion channel embedded in a membrane at resting state. The main structure of a channel is four coiled helices. One of these helices is connected to a hydrophilic negatively charged spiral placed intracellularly. The structures of the four main spirals are loosely bonded with the intracellular surface of the structure and are hydrophilic. The intracellular portion of the structure is negatively charged and polarized toward the hydrophilic spiral.

Any proximate connection between extracellular positive charges and the negative hydrophilic portion will have the electrostatic effect of attraction thus drawing the intracellular side of the structure toward the extracellular surface and deforming the structure. The ion channel is surrounded by a membrane approximately 10 nm thick with the hydrophilic spiral toward the interior of the cell. The pore is effectively formed as a valve blocking ions and importantly insulating against charge, thus preventing electrostatic interaction. Any opening of the pore therefore causes entry Na ions and immediate availability of their charges for interaction. The ionic radius of Na is about 16 nm. The surface of the ion protein channel is hydrophilic, a vertical contraction would be expected and this is in fact what happens (McCusker et al., 2012).

(2) Moment of Threshold:

- As a soliton approaches the ion channel pressure will develop on the sides of the ion channel displacing the helices and removing the electrostatic insulating seal. This produces immediate access for electrostatic attraction between the Na and the negative interior surfaces of the ion channel. Constriction of the central helix produces a mechanical invagination of the four helixes at the pore and simultaneously opening the pore sufficiently to destroy its electrical insulating capacity.
- Positive charges are attracted to the intracellular space but also to the hydrophilic surfaces of the pore. The Na^+ ions are in electrostatic proximity to the negatively charged portions of the structure the moment the electrostatic insulation is broken and are drawn inward.

(3) Threshold Forces:

The incoming Na^+ ions are electrostatically bound to the selector causing an opposing force to the hydrophilic parts of the structure which causes the s6 spiral (for detail see Shen et al., 2017) to be drawn toward the Na^+ receptor. Movement of the hydrophilic s6 portion will undo the main spiral causing the pore to open mechanically in an iris movement (McCusker et al., 2012), which causes a mechanical contraction of the structure. At threshold, the action of contracting the structure from external to internal during pore opening creates forces along the surface of the membrane as the helices are pressured outward. This action is synchronized to the arrival of the soliton and the mechanical energy will be transferred to the soliton, thereby reducing entropy.

(4) Refractory:

When sufficient positive charges have reached the interior equalizing charge adjacent to the hydrophilic spiral, the forces equalize and the pore will close. The pore and structure of the ion protein channel is now at rest. There is now a large concentration of Na^+ ions in the vicinity of the hydrophilic regions and the activating spiral. Any further soliton or disturbance to the membrane will not cause activation of the ion channel pump until the excess Na^+ ions are removed by diffusion. The ion channel is refractory until this charge is cleared allowing for further electrostatic attraction. Synchronization is achieved between the ion channel pump and the soliton and electrostatic force is transferred to mechanical force and then to the soliton.

The APPulse

There is now a large body of evidence showing that:

- a 'soliton' mechanical pulse accompanies an action potential and is stable propagating at constant velocity (Tasaki and Iwasa, 1982; Heimburg and Jackson, 2005; El Hady and Machta, 2015)
- ion channel separation is too great to allow for ion channel interference from adjacent channels caused by ionic charge (Holden and Yoda, 1981; Marban et al., 1998; Catterall, 2012; Johnson, 2015)
- ion channels can be opened by mechanical stimulus (Martinac, 2012; Anishkina et al., 2014; Takahashi et al., 2016; Zhang et al., 2016)
- there is deformation of the membrane by activation of ion channels (Tasaki and Iwasa, 1982; El Hady and Machta, 2015)
- entropy (thermodynamic) measurements do not follow the H&H action potential but do follow the APPulse. (Abbott, 1958; Howarth, 1975; Ritchie and Keynes, 1985; Tasaki and Byrne, 1992; Moujahid et al., 2011).

The action potential measured by H&H is a measure of the sum of all the potential changes of all charges across the membrane over a wide area. The result is always a combination of effects from many ion channels, some open, some closed and some refractory. However, direct mechanical stimuli of axons can elicit action potentials (Howe et al., 1977) suggesting the involvement of a mechanical component (Appali et al., 2012).

The Na^+ Channel Is an Electro-Mechanical Soliton Pump

Positive ions do not behave as electrons and require time and the correct diffusion coefficient to move. Calculation of ion channel distribution from single channel studies, demonstrates that Cable Theory can only account for the action potential in its stable states (resting or maintained by voltage clamp) (Johnson, 2015). The ion channels are spread too far apart and the entropy changes do not match those predicted by the model. The longitudinal resistance in the H&H arrangement is always infinite as there is no mechanism that provides surface spread depolarization. Surface spread and thus speed of action potential must therefore occur by another mechanism. We suggest that this is by a mechanical 'soliton' coupled and synchronized to entropy provided by the ion exchangers described (**Figure 2**). The soliton pulse mechanically opens ion channels leading to further depolarization. Thus a soliton always occurs when a nerve impulse is generated. The membrane soliton is powered by direct mechanical forces from the opening of the pore. These forces originate from charged particles of Na attracted by the hydrophilic units and hydrophilic parts of the structure with electrostatic forces between the Na ions and the negatively charged parts of the ion channel structure. This model accounts for the threshold, spike and refractory period on its own. It produces the correct entropy/time profile if it is assumed transfer of entropy is an almost adiabatic process (in which energy is directly transferred without loss of matter or heat). Furthermore, it explains the ion changes across the membrane. Communication occurs not at the level of the action potential but at the level of ion-channel-pump to ion-channel-pump. In effect computation takes place within the membrane at the rate of transmission. Propagation of membrane pulses is well supported by Mussel and Schneider (2018) who suggest that action potentials may be better described as non-linear acoustic pulses propagating along lipid interfaces and which annihilate on collision, a well-known property of colliding action potentials (Follmann et al., 2015).

Myelinated Fibers

This model also explains action potential transmission in a myelinated fiber where a soliton pulse created at the node of Ranvier, due to a high concentration of ion channels, is then attenuated by the rigidity of myelin. Insulating the movement of the axon membrane has the effect of reducing entropy loss and thus increases efficiency. In a small cylindrical axon, sleeved in myelin, movement is restricted and the entropy created at the nodes of Ranvier cannot be transferred along the axon membrane. Entropy is therefore directionally guided on entry into the myelin sheath creating a pulse-wave within the cytoplasm of the axon. This is similar to the action of pulse wave velocity (PWV), the velocity at which the arterial pulse propagates through the circulatory system. The mathematics is similar to the Moens–Korteweg equation (Moens, 1878) that states that PWV is proportional to the square root of the incremental elastic modulus of the vessel wall given a constant ratio of wall thickness – the myelin sheath. On exiting the myelin sheath, entropy will be transferred back to the axon membrane restarting the APPulse.

Unmyelinated Fibers

The APPulse velocity is a result of the factors contributing to the fluid dynamic qualities of the axon membrane at any point. However, if there are insufficient ion channels producing insufficient energy, then the pulse will not reach subsequent channels and will fail. Thus, in unmyelinated fibers, the action potential travels at a speed commensurate with the membrane dynamics in each part of the axon or neurite. Speed of axonal transmission, and therefore the time impulses take to reach their destination, is variable and depends upon the axon type and diameter (**Figure 2**). Thus, the action potential as expressed by H&H is a measurement of the progression of ionic charge over the axon membrane, it cannot represent the mechanism of propagation.

COMPUTATION BY PHASE DIFFRACTION

In both vertebrates and invertebrates many neurons are multibranched and some have more than one spike initiation zone (Haydon and Winlow, 1982; Ledergerber and Larkum, 2012). This indicates that back-propagation of action

potentials (Stuart and Sakman, 1994) can occur under natural circumstances, allowing action potentials to collide. Deconstruction of the computational variables that can be attributable to the action potential is shown in **Figure 3**: the CAP. Examination of the CAP reveals an inherent ability to compute in a realistic artificial BNN by action of the analog time component of the refractory phase, which is effectively able to reroute (diffract) action potentials along different pathways through the neural network; i.e., the refractory period is capable of interference at axon bifurcations and the axon hillock of cell bodies to produce effective deflection of action potentials along different axonal pathways in the neural network or to cause mutual occlusion.

Annihilation will occur between two action potentials if the refractory period of one action potential interferes with the threshold potential of another (Follmann et al., 2015; Berg et al., 2017; Johnson and Winlow, 2017a). The result of this is that as parallel action potentials meet within a neural network they diffract at branching or junctional points and may be diverted along new pathways. This diffraction occurs due to the timing arrival of the refractory phase in from the point of entry of the action potential into the neural network. The action potential then enters the neural network with a specific refractory period on a timescale defined by the membrane dynamics at that point (**Figure 4**). As the action potential flows along the neurites to the soma it may, at branching points, encounter other action potentials from parallel entry points similarly identifiable by refractory period.

A major advantage of computation with the refractory phase is that along parallel pathways with similar dynamic transmission timing, error is redacted and the pulses synchronize. Over many iterations of nodes this makes the network error and noise free (Johnson and Winlow, 2017a). This is unique to the phase ternary computation and is the first time an error redaction mechanism has been described for a physiological neural network. Error redaction is critical in the smaller neurites of the neural network where signal to noise ratios are of the same order of magnitude as each other (**Figure 5**).

COMPUTATION BY THRESHOLD AND REFRACTORY PERIOD

The threshold is the point at which, either by mechanical (Johnson, 2015) or electrical means (Hodgkin and Huxley, 1952), the Na^+ gates open at a distinct site along the axon membrane. The accuracy of timing in the APPulse can be thought of as the time taken for the threshold to pass between adjacent Na^+ ion channels small neurons where ion channels are separated along the axon as determined by the velocity of the action potential. This distance is from 1 to 5 μm corresponding to a maximum temporal accuracy of about 1 μs if an action potential is traveling at 1 ms^{-1}. The figure of 1 μs can be thought of as the maximum point of accuracy for computation and is a variable figure according to the dynamics of the membrane.

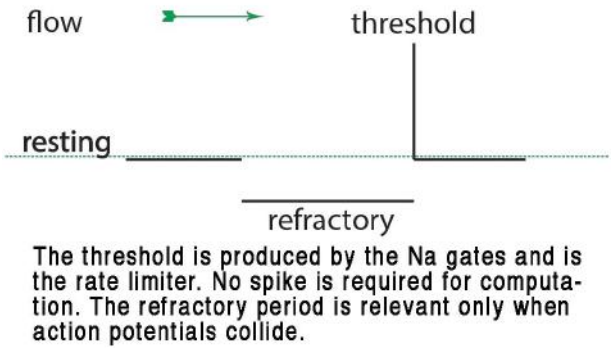

FIGURE 3 | Representation of the CAP, showing the digital resting phase, digital threshold and the analog refractory phase. In this view, the resting potential serves as the ternary 0 of a ternary action potential. Any rise above threshold is the digit or +1 and the refractory period acts as phase -1 refracting any digit+1 during collision to 0. The refractory period is analog and is a result of the specific dynamic of the membrane at that point where the action potential exists. From Johnson and Winlow (2017a) – reproduced under the Creative Commons License.

Inhibition of Incoming Signals by Phase Ternary Annulment

Given that the ternary analog refractory phase often far outlasts the action potential, it is clear that synaptic inputs to a neuron will be obliterated during the refractory period, although powerful inhibitory postsynaptic potentials could prolong it, particularly if delivered during the relative refractory period. The refractory period is a result of protein formation changes and its duration is highly variable. In CAP dependent computation it is the refractory period (defined by the transmission dynamics of the point of interaction, and thus a non-linear time variable) that defines computation. In both the H&H model and the APPulse, the ion channels are inactivated during the refractory period. The refractory period cancels adjacent, out of phase, action potentials when they collide annihilating one of them to produce a change in phase. A neuron is thus capable of taking distinct all-or-none action potential inputs in specific temporal phase and, by interference, changing the phase to create distinct all-or-none action potential outputs. Each neuron is therefore capable of fulfilling computation independently.

NOISE REDUCTION

The usefulness of artificial-networks to model activity is limited by the amount of noise and spontaneous activity in biological systems and synaptic studies give little insight into conduction in more highly evolved neural-networks, where axon conduction is diverse and seemingly unreliable with an alarming amount of noise (Bullock, 1958), which must be reduced for any neural

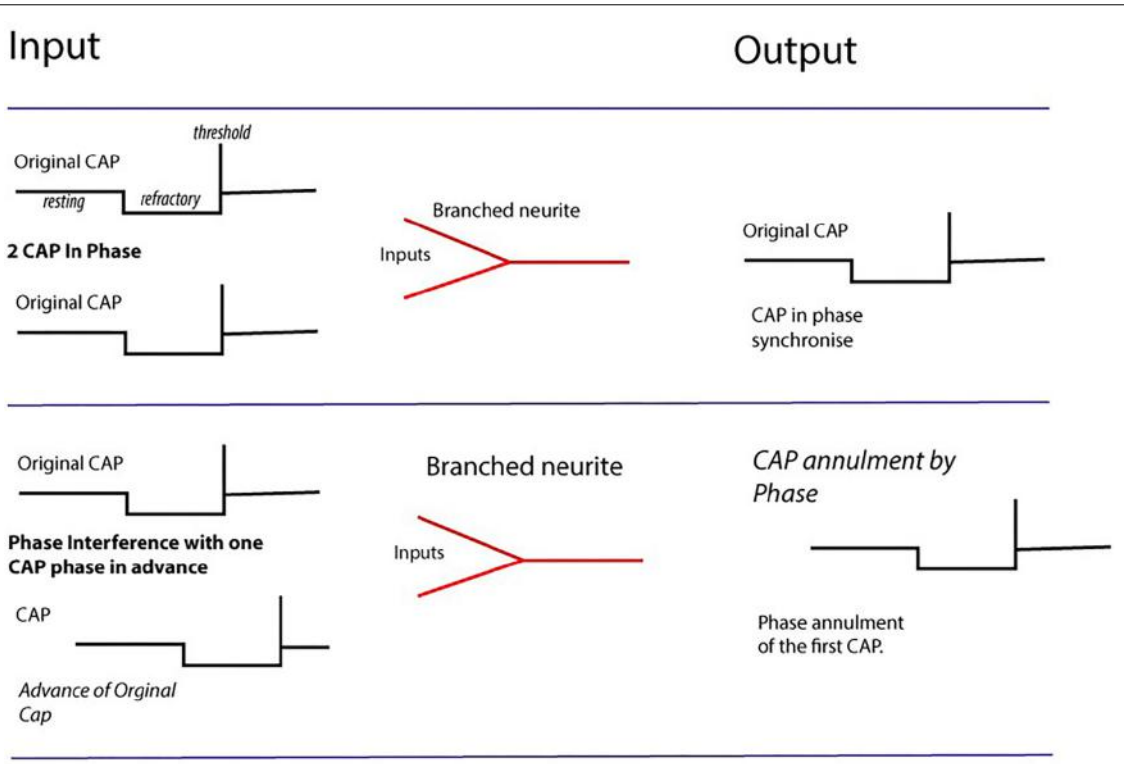

FIGURE 4 | Computation by phase ternary interactions – Collisions from CAPs may result in nullification of CAP (second row) depending upon phase and the dynamics of the membrane at the point of collision. Multiple collisions form patterns navigating pathways through the network. Action potentials firing in-phase map together, whilst those with +1 and –1t overlapping cancel to 0.

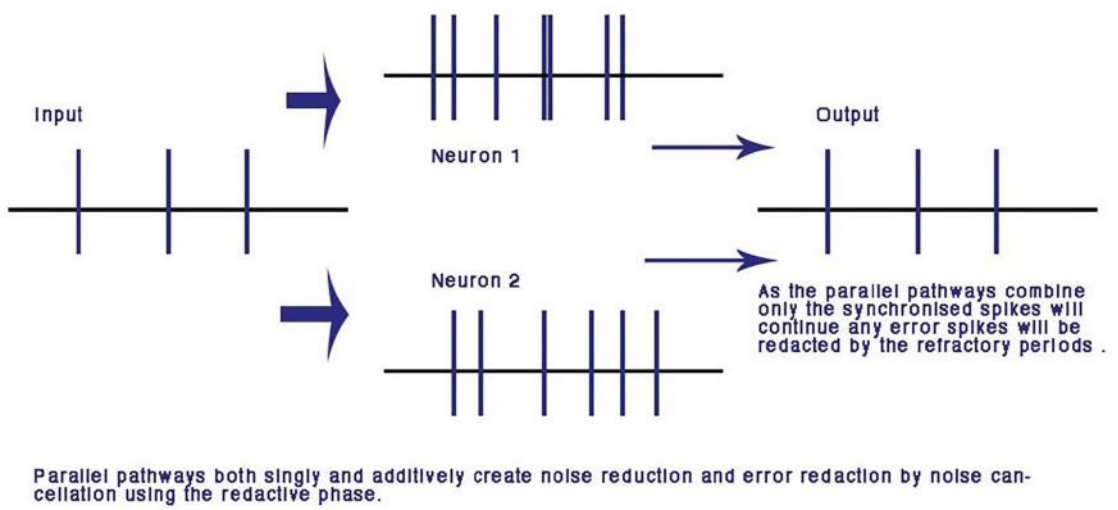

FIGURE 5 | Error and noise redaction by phase ternary computation. In parallel connections, any out of phase errors and spontaneous spikes are automatically redacted by the refractory periods of neighboring spikes: using the CAP any phase shift or spontaneous spike will be reduced to the resting potential by the colliding neighbor CAP. From Johnson and Winlow (2017a) – reproduced under the Creative Commons License.

network of depth. The parallel processing of axons leading to the same neuron reduces variability in the CAP neural network where connections are randomly formed and neurons are connected by more than one pathway and may reduce signal to noise ratio (**Figure 5**). In a concatenated balanced phase system with many interfering CAPs, noise is automatically negated at each parallel point of processing by interference. Error redaction occurs where pulse trains travel along adjacent parallel pathways

whether through one or more neurons or points of contact. This is a mathematical certainty where two CAPs collide from parallel pathways to a common node the trailing refractory period will annul the second threshold. This error negation is particular to phase ternary and depends upon the logic of interference where two spikes are compared from different sources and tested automatically for a match. Error in this system is negated at the point of balanced phase computation and activity within the network becomes synchronized.

CONCLUSION

- We have combined the Hodgkin-Huxley model of the action potential with the soliton theory to produce a unified model of action potential propagation, the APPulse, which also applies to cardiac action potentials. This model is not restricted to spiking neurites and can be applied to non-spiking neurons and any active membrane consisting of ion channels.
- The Hodgkin Huxley model informs only the level of progression at the macroscopic level, but not the underlying mechanical processes. In actuality action potential propagation is due to a combination of separate *microscopic* elements: the APPulse, an electromechanical mechanism incorporating a soliton-ion channel pump, which produces a phase ternary CAP from the separately identifiable resting, threshold and refractive phases. Recovery occurs when the Na^+, K^+ and other ions re-establish membrane stability.
- Phase-ternary computation within physiological neural networks is fast, accurate to microseconds, and efficient. It diffracts parallel inputs within a network along pathways defined by the phase in which action potentials arrive at the neural network in temporal synchrony. In contemporary computing parlance: phase-ternary computation is the brain's machine language and is capable of storing information regardless of any other memory storage or retrieval processes within that network.
- In the H&H action potential the temporal accuracy of the point of computation is variable, restricted to accuracy estimated from the H&H curve. Computation is only accurate to calculated milliseconds. By contrast computation with the action potential pulse is accurate to the exact threshold distance between specific ion channels in microseconds along an unrestricted neurite giving 1000 times greater computational precision.

AUTHOR CONTRIBUTIONS

AJ: the original concept. AJ and WW: split the writing of the review about 50/50 with much discussion between us about the way to finalize the article.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.2 William Winlow and Andrew S Johnson. ***"The Action Potential Peak is Not Suitable for Computational Modelling and Coding in the Brain".*** EC Neurology 12.4 (2020): 46-48

Action potentials are highly plastic phenomena and vary greatly in trajectory from neuron to the next. The temporal positioning of the spike peak is very variable and modifiable by synaptic inputs. Consequently, it is inappropriately used in binary computational models of neuronal activity. Here we demonstrate that the action potential threshold has temporal constancy and should be used in ternary computational models, whose phases are: resting potential, threshold, and the time dependent refractory period, which is an analogue variable. Thus, the action potential threshold is the most appropriate temporal fixed point for computational modelling, not the action potential peak.

Computation between neurons is temporal and uses frequency to determine a result. We describe how this takes place in context to our and other studies.

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EC NEUROLOGY
Mini Review

The Action Potential Peak is Not Suitable for Computational Modelling and Coding in the Brain

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Abstract

Action potentials are highly plastic phenomena and vary greatly in trajectory from neuron to the next. The temporal positioning of the spike peak is very variable and modifiable by synaptic inputs. Consequently, it is inappropriately used in binary computational models of neuronal activity. Here we demonstrate that the action potential threshold has temporal constancy and should be used in ternary computational models, whose phases are: resting potential, threshold and the time dependent refractory period, which is an analogue variable. Thus, the action potential threshold is the most appropriate temporal fixed point for computational modelling, not the action potential peak.

Keywords: *Action Potentials; Resting Potential; Threshold; Computational Fixed Point*

Introduction

Action potentials play a number of roles in the nervous system, but are primarily thought of as the means by which cellular communication takes place between neurons and serve to trigger secretions from nerve terminals. Binary computational models of nervous systems usually use the peak of the spike to initiate activity, but this is an inaccurate method of computation, given the plasticity of action potentials not only in cell bodies[1,2], but also in nerve terminals [1,3,4] and axons [5]. Action potentials are generated by powerful ionic driving forces created by metabolic pumps such as the sodium-potassium pump, which instigate the membrane potential, but their properties vary substantially from one neuron to the next [6,7].

We have shown elsewhere that ternary phase computation is much more appropriate in modelling nervous activity where threshold is the instigator of the computational action potential (CAP): the three phases are thus: resting potential, threshold and the time-dependent refractory period, which is an analogue variable [8-10]. In particular the variable position of the action potential peak is well documented [3,7] and the maximum rates of depolarization ($\dot{V}_d$) and repolarization ($\dot{V}_r$) are highly variable phenomena and are clearly frequency dependent (Figure 1). This can be demonstrated using the phase plane technique (Figure 1C) [11], which is very useful for determining the threshold of action potentials [7,12-14]. Frequency changes result in a shift of the action potential peak and both $\dot{V}_d$ and $\dot{V}_r$ are modifiable by excitatory and inhibitory synaptic inputs [1,3,7].

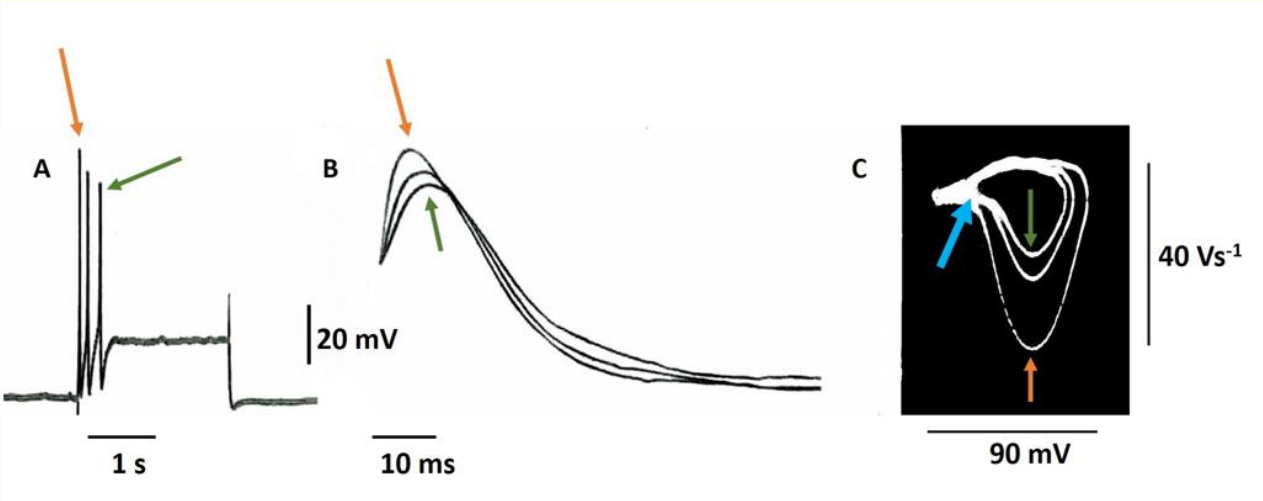

Figure 1: *Plasticity of action potential shape and action potential peak recorded from the soma of a fast adapting pedal I cluster neuron (for details see 15) in the intact brain of the mollusc Lymnaea stagnalis (L.). The cell was normally silent and activity was initiated by a 0.2 nA current pulse of 3s duration injected into the cell via a bridge balanced recording electrode. The same three spikes are represented in each case; a) on a slow time base, b) on a faster time base and c) as a phase plane portrait in which rate of change of voltage (dV/dt) is plotted against voltage itself and the inward depolarizing phase is displayed downward maintaining the voltage clamp convention (see 11 for details of the phase plane technique). In each trace the peak of the first action potential is indicated by an orange arrow, the second action potential peak is unlabelled the third action potential peak is indicated by a green arrow. The three successive spike peaks clearly vary temporally from one another, but the threshold point of initiation remains constant as indicated in c) by the blue arrow in the phase plane portrait (Data previously unpublished but provided from William Winlow's data bank).*

Figure 1 clearly indicates the temporal variability of the action potential peak (V̇d) and the temporal constancy of the action potential threshold. This supports our opinion that threshold is the most appropriate temporal instigator of the computational action potential [8-10].

Conclusion

Future modelling of neural activity should use phase ternary computation, where the second phase of computation becomes the action potential threshold, the fixed point of action potential initiation.

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5.3 *Johnson AS, & Winlow W. (2019).* ***Are Neural Transactions in the Retina Performed by Phase Ternary Computation? Annals of Behavioural Neuroscience****, 2(1), 223-236. https://doi.org/10.18314/abne.v2i1.1893*

This paper deconstructed the computational mechanisms of nerve impulses withing the retina demonstrating that the retina computes using phase ternary computation. We showed that binary spikes cannot work to compute the retina in sufficient time and provided a corrected model for functional computation in the retina with evidence.

This is a very important paper in that we take the circuit diagram of the retina and apply phase ternary computation to mapped neurons using APPULSE to show that the relational computation between the light receptors and the basal ganglia is phase ternary computation mean frequency computation. This is first time this has been demonstrated and confirms both that the APPULSE should be considered the purveyor of information but that also the retina computes by phase ternary computation.

In addition to this work, I also discovered similar evidence for mean frequency computation in the ear and in sensory nerve ganglia. By extension the rest of the brain computes by phase ternary.

An introduction is given to quantum processing and this paper also contains elements general to the theory of computation.

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Gratis

Review Article

Annals of Behavioral Neuroscience

Are Neural Transactions in the Retina Performed by Phase Ternary Computation?

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Abstract

Substantial evidence has accumulated to show that the action potential is always accompanied by a synchronized coupled soliton pressure pulse in the cell membrane, the action potential pulse (APPulse). Furthermore, it has been postulated that, in computational terms, the action potential is a compound ternary structure consisting of two digital phases (the resting potential and the action potential) and a third-time dependent analogue variable, the refractory period. Together, with the APPulse, these phases are described as the computational action potential (CAP), which allows computation by phase. The nature of transmission, and thus computation across membranes, is dependent upon their structures, which have similar components from one neuron to another. Because perception and therefore sentience must be defined by the capabilities of the brain computational model, we propose that phase-ternary mathematics (PTM) is the native mathematical process underlying perception, consciousness and sentience. In this review, we take the CAP concept and apply it to the working of a well-defined neural network, the vertebrate retina. We propose an accurate working computational model of the retina and provide an explanation of computation of the neural transactions within it using PTM, and provide evidence that could form the basis of understanding neural computation within the entire nervous system. Evidence is presented of phase ternary computation (PTC), defined in phase ternary mathematics and shows an exact mathematical correlation between the activity of the amacrine cells, the bipolar cells and ganglion cells of the retina, once these cells have been activated by light falling on the cones. In this model, the computation of luminosity of multiple cones synapsed to a bipolar cell is performed by phase ternary mathematics at the points of convergence of CAPs. Redaction by the refractory periods of converging CAPs eliminates all but the leading APPulse resulting in sampling and averaging. In phase ternary analysis (PTA), the physiology of synapses defines their primary action as latency changers, changing the time taken for impulses to travel between points of convergence. This paper describes a novel type of computation, PTC, with evidence that it is the fundamental computational method used by the retina and by association the rest of the brain. By comparing the morphology of neurons it is now possible to explain their function singly and in networks. This has profound consequences both for our understanding of the brain and in clinical practice.

Keywords: Retinal coding, Retinal neural network, Phase ternary computation, Computational action potential, Neural transactions, Action potential pulse, Timing by synchronization, Error correction, Mean sampling, Computation of luminosity

Introduction

The basic circuit diagram and physiology of the retina are well researched and the activities various components are now known in response to light. We know from experimental evidence that computation of patterns of activity from 130 million light receptors are coded into 1000,000 visual neurons [1,2] so that the image is perceived correctly in the visual cortex at different light intensities. This is also a huge reduction from input to output requiring error free efficiency of computation, which has to be accounted for. In the retina there is no clock or method to synchronize to the natural brain neural network (BNN) but coding takes place accurately and efficiently and there is probably only a single mathematical method for this to take place for the network to remain error free.

For synchronization to occur in the retina there must be a mechanism inherent in the action potential to coordinate activity and this can only occur at the convergences of the action potentials themselves. This requires a mechanism to correct the temporal relationship of one action potential to another. This is not possible with a binary action potential based upon a spike but is possible with the computational action potential (CAP). This is a phase ternary pulse based upon the threshold, refractory and resting states. The importance of phase ternary computation (PTC) rather than binary computation within the nervous system has been dealt with elsewhere [3].

Binary computation was primarily accepted because of the inexpensive ease of manufacture of binary transistor chips and research has assumed, without evidence that the computational parameters must exist to service binary computation within a physiological BNN. Knowledge in the fields of computation and AI has diverged from neurophysiology and physiologists now have intimate knowledge of the action potential at the site of the membrane in terms of both transmission dynamics and activity of the soliton pulse. Recently we [3] deconstructed the action potential and APPulse into the computational elements of threshold (defined by the moment of opening of the Na channels) the refractory period and the resting period; these were notated in balanced phase ternary as 1,-1, and 0 respectively. The APPulse [4-6] and the Computational Action Potential CAP requires only single action potentials to compute in parallel. Hodgkin Huxley Cable Theory [7] may also be thought of as a description of PTC because in their paper they describe spike, refractory and resting phases. However, Cable Theory cannot work in the retina because computation at the timing precision of more than 1ms is not possible in the retinal network. Therefore, timing would have to be redacted by other mechanisms. In artificial neural networks this error is adjusted for precision artificially, e.g. by introducing temporal coding of spikes into the model [8] but there is no evidence from the histology or the physiology of the retina that such an equivalent biological system exists.

Of paramount importance in consideration of computation in the nervous system is the mechanism by which individual action potentials interact. In artificial intelligence (AI) models, interaction takes place at gated nodes (Figure 1d). In a brain neural network these are replaced by the synapses, points of convergence and divergence (Figure 1a,b and c). At synapses the computational dynamics of the CAP terminate, until a new CAP is triggered postsynaptically [3]. Thus, the coding within the synaptic space is essentially binary in that an incoming spike may or may not trigger an outgoing spike. In a sense synapses play a secondary, but very important role in PTC, by altering the latency of postsynaptic responses. Long-term synaptic modifications may still occur to modify the output of the system. Contemporary understanding of computation in a computational or physiological BNN is limited to the ability of synapses to modify the properties of their connections according to the previous stimulus. Many models have been created for computing for example spike-timing-dependent plasticity [8] both in neuroscience and in AI: all use calculations of temporal positioning calculated from binary spiking neurons. PTC simplifies this process by eliminating the need for additional complexity.

Mechanical surface waves are known to accompany action potential propagation [9] in the form of solitons [10]. Coupling of the mechanical activity and Hodgkin Huxley Cable Theory have been suggested [10].

We have recently postulated that action potentials take the form of an APPulse i.e. an action potential coupled with a soliton in the nerve membrane [5,6], rather than the more orthodox view that the mechanism of regenerative action potentials can be explained by Cable Theory. In our view, the APPulse mechanically deforms membrane ion channels allowing entry of Na^+ into the cell, which then reaches threshold to trigger a regenerative action potential. This is critical to a computational analysis of functional neural networks in the brain, which will in future be important in the development of artificial

intelligence (AI) because realistic models of neural coding in the nervous system need [11] first to be proposed, tested and understood.

Timing in a neural network

Intrinsic to AI is the belief that gated timing of synapses synchronizes computation. Where computational models have been proposed [12], models that attempt to use conventional computer theory conflict with the known neuronal morphology and function. In these models the timing of action potential and computation through the network is synchronized as a conventional computer by clock. We know that "Neuronal morphology and function are definitely interrelated" [13] indicating differing latencies and transmission behaviors for morphologically different neurons. In the retina timing of action potential from one point to another depends upon its transmission speed and the length of the neuron – both of which are variable from one neuron to the next. The neuronal connections in the retina do not permit a Turing based machine. There is no functioning clock where timing of computation can be coordinated in the retina and yet sight is extremely accurate robust and error free. The only model that will sustain computation in the retina is by synchronization and error correction using the exact physiology described by neuronal connections physiology and morphology. This is the model proposed by the CAP.

The CAP demonstrates that the action potential is capable of synchronizations if its phase ternary mathematics are calculated over the network. Thus, timing elements are unnecessary in a synchronized system. Basic computation requires that input be transferred to output in a logical manner with timing between input and output fixed. Timing is critical in a BNN and just as importantly the precision of timing of a pulse allows us to judge its constituent mechanisms. Computation is not just in phase but it is in temporal phase. In a neural network the rules of parallel processing are considerably different for PTC as compared with binary computation. Binary networks must be gated but a phase ternary network synchronizes signals (Figure 1).

In the Lateral Geniculate Nucleus 1,000,000 distinct parallel inputs from the optic nerve merge into a connected network simultaneously [1,2]. To distinguish between the activities of 1 input from 999,999 others requires a sufficient temporal gap. The combined activity forms the complete image that we see. If 1,000,000 inputs converge to the same network (effectively layer t1 of Figure 1d), each separate input must be able to be distinguished from all others. For any individual impulse to be distinguished against all other parallel inputs temporally, each second must be divisible by the number of inputs such that the precision of timing per second can be distinguished. This produces a figure of 1,000,000 Hz, indicating an ideal precision below 1 microsecond. This is a basic calculation where error and duplication have not been considered and which would increase the computation time. Cable Theory cannot give this level of precision, but the APPulse is estimated to give a precision of 1.5 microseconds based on the distances between ion channels [6].

It is widely accepted that light reactive cells increase frequency of discharge and importantly we know that Retinal ganglion cell (RGC) frequency of discharge is proportional to mean light over RGC receptive fields [14,15]. Activity of the different light receptors has been evaluated [16] and their connections have been traced. Retinal ganglion cells are connected via bipolar cells to many light receptors. Luminosity across the light receptive area instigates activity in its respective retinal cell ganglion. Many light receptors are connected in parallel with different sensitivities to light and will have a range of activities so that as luminosity changes, then so will their combined activity. These parallel frequency differences must be collated to ensure that their mean frequencies represent the bipolar cell frequency, thereby generating meaningful information.

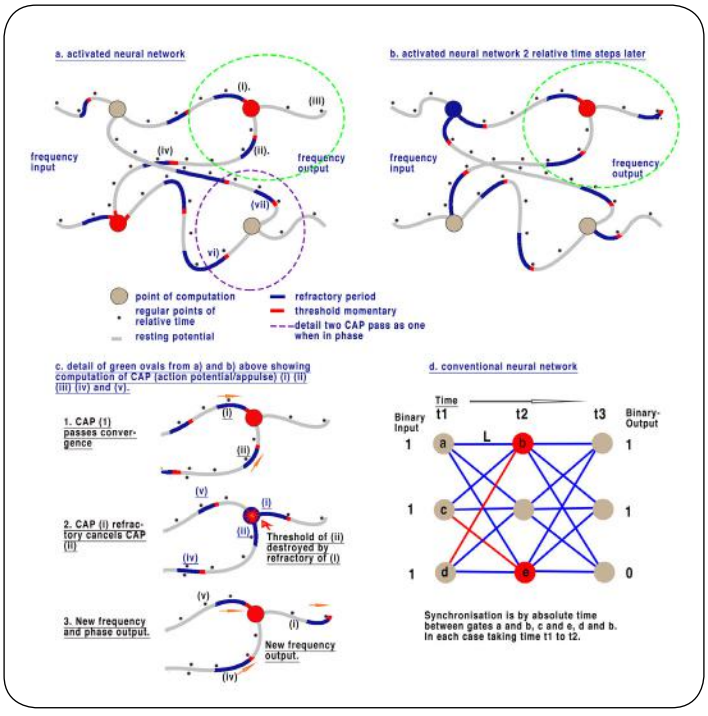

Figure 1: Simplified comparison of computation in a contemporary artificial neural network with a

physiological brain neural network utilizing phase ternary mathematics.

- a) and b) are illustrations of the connectivity of neurons within a physiological brain neural network modified to represent the Computational Action Potential CAP [3] with detail c). d) Is a comparison with a contemporary artificial neural network. In a) and b) action potentials have different latencies as they travel from node to node. Distances between nodes are also different. Points of connection are represented by large brown dots, these are the points of convergences. Impulses travel from left to right as illustrated in red by the threshold and in blue the refractory following the rules of the CAP. Synapses lie between points of convergences and are excluded from the diagram for simplicity; their action in computations is discussed later. There is no system of absolute timing and action potentials arrive according to the timing of their respective neurons. In figure 1 a) (i) the threshold of a CAP arrives at a convergence and passes while the threshold of (ii) is annulled resulting in the phase shift shown in b) two relative time steps later. c) The three steps are shown in detail. Successive phase shifts change the frequency of the CAP resulting in unique computation from phase. In both figure 1a and b signals must be synchronized, in an artificial neural network this is done by timing gates, however no timing exists in the brain to facilitate such a synchrony. Binary does not synchronize but phase ternary does. In a) b) c) and d) all inputs must synchronize. The precision of this input in d) depends upon the timing between t1 and t2 and the gating. This timing can be thought of as the precision of the network. In the brain neural network this precision is dependent upon the absolute timing of the threshold.
- c) Detail of green oval region showing three successive moments in relative time marked by small dots. Note CAP (ii) is cancelled changing the output phase of (iv) and affecting the overall frequency. Figure 1a Purple oval in contrast shows two CAP (vi) and (vii) converging together in temporal phase and pass as one. These diagrams illustrate the basis of phase ternary computation: in a neural network containing many inputs and many outputs specific phase temporal patterns are created representing input. This process is described in detail for the retina in figure 5. Computation is therefore a function of all the frequencies from all input neurons simultaneously.
- d) Diagram of a simple contemporary artificial neural network where information is gated in absolute time. In contemporary AI, inputs must code for output in a logical fashion, these are usually represented in binary as 0 and 1. AI assumes that computation is by gated synapses. In addition information passes through the network in an orderly fashion with points of computation t1,t2 and t3 synchronized by timing. In such a model latencies are assumed fixed.

The basic network theory is similar in a b c and d in that unique input is expressed as a pathway through the network to unique output. The difference is that the phase ternary arrangement in a,b, and c) is that at each point of computation/convergence nerve impulses are synchronized against each other therefore redacting error from each layer. The processing of parallel information across multiple threads of information is therefore temporal, dependent upon the minute changes in phase of parallel impulses producing changes in frequency of successive individual CAP. The nodes for CAP computation are the bifurcations and convergences of the neurites, which may also be on postsynaptic membranes. Importantly inputs are not represented by binary but by frequency of impulses assessed from the temporal relationships between thresholds in phase ternary.

All computations that take place compute changing frequencies, there is no evidence of a clock. In figure 1d, a conventional network illustrates defined binary output representative of the binary input; in figure 1a action potentials that enter the network are computed to give specific frequency characteristics of outputs and are shown as CAP with a threshold in red followed by a refractory period in blue, the axons have different timings, latencies and have speeds according to the membrane components. There is no timing possible over the network – the only reliable measurement is the latency from receptors to RGCs. Error must therefore be inherently and simultaneously removed. There are no mechanisms available within the eye for error or noise correction so the only possible method is from synchronization as action potentials travel through the network. There is no present mechanism from computer science or AI that can explain this.

Phase ternary analysis of retinal coding

We postulate that synchronization of activity in the retina is via the CAP when considering the structure of

the retina (Figure 2) and the accuracy of visual processing from the receptors cells to the retinal ganglion cells. Although the action potential and the APPulse have ternary mathematics comprised of the resting threshold and refractory periods, their computation processes are different: action potential computation must be calculated from the spike, which is temporally inaccurate, while the CAP is calculated from the threshold. The CAP can be expressed mathematically in balanced phase ternary as 0 +1 and -1. When two physiological action potentials collide they annul due to the two refractory periods. This is illustrated in figure 1c. If two action potential converge in the same direction the result is always addition of the ternary phase in which they arrive, this depends upon the timing of the CAP and the latencies of the neuron (Figure 1a). The timing of computation is critical and as no clock exists in the CNS capable of absolute timing, activity of impulses must be synchronized.

For computation to take place in a neural network, inputs into the network must match outputs in a logical manner (Figure 1a and d). In the retina the activity of the light receptors is represented as the output of the RGCs to the optic nerve. In contemporary binary computing these values are represented as 0 and 1 where 0 is resting and 1 is the spike but in PTM they are expressed as 0 +1 and -1 representing resting, point of threshold and refractory respectively, i.e. PTM is defined by the phase structure of the CAP. Note that the refractory period in the CAP does not match traditional ternary mathematics: in the CAP the refractory period always returns -1 when in collision with another CAP and is a distinct variable time related to each point of convergence.

In the visual system, the coding of parallel information commences in the connections between the light receptors and RGCs. Early studies suggested that RGCs are the first clearly spiking neurons, while none of the other retinal neurons were believed to conduct regenerative action potentials [17,18], although amacrine cells were thought to carry dendritic spikes under some circumstances. Over time this view has been called into question and it is now known that amacrine cells can conduct action potentials [19,20] as can some classes of bipolar cells [21,22]. The implication of these findings is that it is now possible to analyze the visual system all the way from the amacrine and bipolar cells using phase ternary mathematics. It should be noted that the APPulse computes using PTM [3] and the soliton will do so in non-spiking neurons, where the threshold is calculated from the soliton activating the opening of ion channels. This is independent of spikes and is ideally suited to calculations on non-spiking neurons.

The retina uses parallel pathways to encode different features of the visual scene according to the activated state of the parallel pathways. These distinct pathways converge on circuits that mediate a distinct computation allowing for precise sight. In all likelihood, there is only one solution to how the retina codes. The prerequisites are that the changing frequency of parallel inputs from the light cells must be reflected in the changing frequency of parallel outputs in a logical manner so that error is redacted and all information is transformed synchronously. On this basis, we propose that a computational action potential pulse (CAP) [3] is the most appropriate analytical tool for evaluation of network models both in artificial physiological BNNs such as the retina, a peripheral extension of the brain.

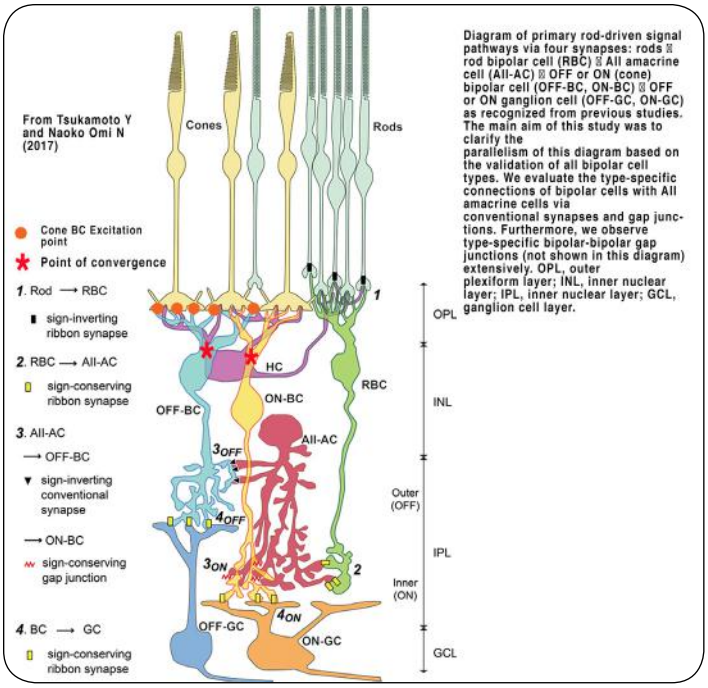

Figure 2: The layout of the retina.

To evaluate the computational method used between the cones and the bipolar cells shown as OFF-BC and ON-BC. The rates of firing are known for both the cones and the bipolar cells and the contrast activity frequency response at the exit of the bipolar cell is known to evaluate to the average firing rate over time [15]. Using this knowledge, it is possible to mathematically model the exact mechanism that achieves this computation in the bipolar cells. (Modified from Tsukamoto and Omi [23] under the Creative Commons License (CC BY)).

Synapses and latency

Synapses are conventionally assumed to act as binary gates in neural networks with changing activity altering their transmission properties [24]. Computation has been assumed to take place at the synapses and no further investigation has taken place to examine other forms of computation. Microscopic examination shows that synapses, although closely spaced, separate neuron from neuron and their primary purpose is to modulate the activity of the post synaptic membrane affecting latency, leading in many cases to an action potential. The timing latency involved in the activation of the postsynaptic membrane is fast and is the primary function and depends upon transmitter as discussed in our previous work [3]. Any further chemical activity caused by the release of neurotransmitter will be secondary and will only affect other nearby neuronal membranes after the passage of the APPulse. Just as with CAP convergence [3] activation of the membrane is the first activity of synapses and the refractory period will block any further CAP at the point of connection between the synapse and the membrane. Either synaptically derived or neurohumoral chemical interference is considered secondary and affecting frequency due to modification of the refractory period.

In PTC the activity between two post synaptic synapses converging on the same membrane is determined by the change of phase generated by the different latencies of synapses as they pass a point of convergence and to the transmission latency of the neuron. If they are both in phase both CAPs pass, but if either is delayed the first will cancel the second. This is illustrated in figure 1a, b and c. Synapses in this model affect the latencies of the transmission and cause inhibition by changing phase of one or both CAPs. Two pathways containing different neurotransmitters will have different latencies dramatically changing computation. This computation takes place by single action potential and is precise. Neurotransmitters all have different release latencies and response latencies. A neural network with more than one neurotransmitter will have differing latencies within it. Latency changes may also occur due to external hormones, which compete non-linearly with neurotransmitters. Finally, latency is also a product of the transmission dynamics of neurons. Any chemical interaction can therefore only affect subsequent frequency after the initial CAPs have passed because the CAP is produced first and travels at a faster speed than diffusion.

The retina is a regular array of neurons set in defined patterns and layers. In this array, synapses evidently have important functions in computation and the phase ternary model proposes that synapses act as latency changers to the phase computation as well as inhibitors. Synapses therefore have the ability to change a CAP ternary phase changing the computation at the convergences of connections and changing the direction of travel of CAP along neurites. Any error at synapses is redacted by synchronization. We have addressed the important issue of error redaction elsewhere [3,6].

Computational coding of retinal signaling

Cone photoreceptors are reactive to bright light of different wavelengths, depending upon their type, according to the structure of the visual pigments or opsins in their outer segment regions [16,25]. Cones have been categorized into many types [26]. Some are hyperpolarized by light and some also depolarized by dark [27], and a release of glutamate at the cone output synapse, the cone pedicle, transfers this signal to the postsynaptic neurons.

The molecular mechanisms of photon-transduction to release of neurotransmitter are similar in rods and cones, but the quantitative details of the function of each of the biochemical and biophysical processes in this pathway differ between receptor types [16]. Information is processed in parallel by the structured network of the retina in a feed forward from the rods and cones to RGCs from where they project to the optic nerve (Figure 2). The timing and output from the basal ganglion cells have been known for some time, but more recently the cones and the bipolar cells have been extensively studied for connectivity and activity. Information moves progressively from the cones to the RGCs. The total information represented by the RGCs over time therefore reflects the information from the rods and cones over the retina.

Cone types are reactive to contrast banding, the activity of each type depending upon the level of light within that band [14,15,28]. The organisation of the cones in relation to the bipolar cells has been studied [26,29,30]. Connectivity is complex but there are rules to the network. Bipolar cells are arranged in overlapping cone receptive fields [23,31]. The output from the cones to the bipolar cells and to the individual RGCs is known, as are the relative numbers of connections. Individual cone activity is frequency dependent upon luminosity within cone-type bands. The frequency of output of action potential from the RGCs is proportionate to light

contrast at the cone receptive fields [14,15] for each type and are similar in frequency to the output of the cones.

In the case of the retina, much of the information processing is performed through gap junctions and neurons that do not spike [32]. Gap junctions have a shorter latency than chemical synapses [33]. The CAP can therefore be represented in spiking neurons by either the action potential or APPulse but in non-spiking neurons we conjecture that graded activity may also be accompanied by the by a soliton wave to allow opening of membrane channels.

The mathematical derivation of mean sampling from phase ternary analysis

Cone-bipolar cell connections: Recordings from RGCs of the activity of single bipolar cells demonstrate computation when multiple cones are activated. The result is temporal activity related to mean field luminosity [14,15]. The ranges of frequencies detected in output were similar to the input. The calculation of luminosity change across the cone receptive field by the bipolar cell is therefore calculated by the change in frequency, at any moment, of single action potentials compared with the mean. This computation of average luminosity across the RGC receptive field must take place before the RGC as the connection between the bipolar cell and the RGC is one to one (Figure 2). This is a mathematical network template and can be used to resolve the computational functioning of the bipolar cell. Individual cones are responsive to light over the range of that type of cone [25]. There are two sites of interest on the bipolar cell where interaction may take place, within the synapses themselves or where they converge on the post-synaptic membrane, i.e. on the RGC.

Bipolar cells encode diverse temporal image signaling from the cones in a subtype-dependent manner to initiate temporal visual information-processing pathways [14]. Cone bipolar cells individually react to luminosity by increasing frequency (f) of action potentials (i) [14,15,34] within the bandwidth of the cone type [14]. Figure 2 illustrates the distance between cone-bipolar cell (BC) activation points and the point of convergence. Computation cannot take place from one synapse that would affect another except by CAP due to the distances between multiple cone/bipolar synapses and their interaction points. A graded potential or action potential, depending on bipolar cell type, precedes any possible chemical interference between the separated cone-bipolar cell synapses that converge and this will proceed towards the RGC before any intra-synapse activity. The point of convergence and computation is therefore the site at which the outputs from the cone/bipolar synapses connect (Figure 2 Point of convergence). This membrane is situated some distance proximal to the synapses. In this case, the action of synapses in PTM is to depolarize the membrane changing it to a refractory state and simultaneously adding latency to the action potential. Latency is defined as the amount of time necessary to pass between convergent/divergent points in the network. The result during convergence of CAPs is to continuously sample the frequency change from the mean (Figure 3).

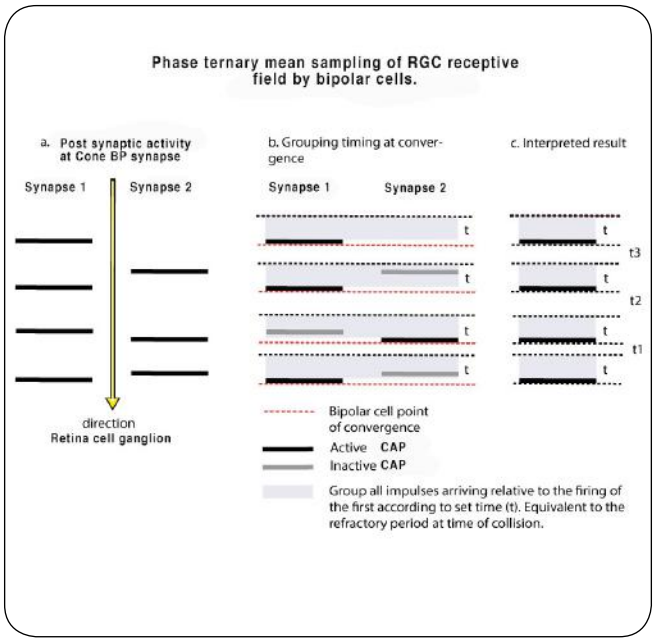

Figure 3: Mean sampling. The output frequencies from two cones converge at the point of convergence of a bipolar cell. a) Represents the CAP travelling from the Cone BC synapse and the point of convergence (red) on the membrane as shown in b. Each red line represents the same point of convergence as successive CAP converges. The first CAP inactivates the whole of the membrane for the refractory period annulling any within the sampled refractory time. Here activity is sampled into groups of time t. Time t is a set period of time after a CAP reaches the convergence of the bipolar cells where the activity of all the cones are combined. (t) Represents a grouping of Cone BP 1, cone BP 2, etc. Any impulses arriving within the grey refractory area are inactivated by the membrane refractory period. c.) Shows the interpreted result after the pulses within the selection area are redacted and represents the information passed to the RGC. The pulses in the interpreted result have a fixed time t and each has a variable time indicated as t1 t2 t3 samples of frequency

change from the previous. t1 + t2 + t3 etc. sampled by time represents the mean of light focused on the cone receptive field. If many cones are arranged in parallel Cone BP 1, cone BP 2, Cone BP 3 etc. The mean of, t1 t2 t3 will be the mean luminosity of light over that time period. The accuracy therefore of calculating the mean depends upon the timing of the refractory period. From this we can see that the output from the bipolar cells to the retinal ganglion cells is a mean value of the added frequencies from its input, achieved by mean sampling at the point of convergence. The first CAP/ action potential will pass this point and leave a refractory period blocking any subsequent activity. This results in the mean sampling of successive CAPs originating from different light receptors. The refractory period is a product of the ion channels and membrane components and is thus stable. This refractory period measured in absolute time deselects successive bands of CAPs, which results in the time difference between these selective bands being a mean sample of the input. This can be expressed as:

$$m^r = \frac{(\sum^r f^r)}{n}$$

Where m^r is the resultant sampled mean from the collisions, f^r is the count of impulses arriving from each light receptor during the refractory period r. within each suppressed refractory period; ∑r fr is the summation of this number, n is the number of convergent light receptors. The time between r and the next impulse is dependent only upon the timing of the next light receptor t1, t2, t3 as shown in this change in time t^n between refractory periods is a measure of mean frequency change.

BP = bipolar cell.

Computation of mean frequency by the bipolar cells revolves around there being only a threshold followed by a refractory period. It is equivalent to mean sampling of the frequencies as demonstrated in figure 3, i.e. the output from the bipolar cells to the retinal ganglion cells is a mean value of the added frequencies from its input. This is achieved by mean sampling at the point of convergence. This method of evaluating mean sampling fits with the precise definition of phase ternary described in [3,4,6]. This way of computation is only possible between single action potentials in parallel, because there is a defined threshold with precise timing of less duration than the refractory period. It is impossible to use a spike-timed action potential for this computation, as accurate timing of the spike to less time than the refractory period would need concurrent computation for which there is no evidence. Phase ternary mean sampling is expressed mathematically as the grouping of phase ternary activity at an interaction point followed by addition. As yet there is no other known mechanism that can explain the mathematics as resolved from experimental data, the physiology and connections of the retina. Although the CAP can be formed from the Hodgkin-Huxley Cable Theory, for frequency mean sampling to take place each CAP must have a defined beginning such as a measurable threshold.

The Computational Action Potential [3] simplifies the mathematics by recognizing that each CAP may be in three states, resting, threshold, or refractory. For a CAP to pass during convergence the threshold timing on convergence must be synchronized with another CAP in phase or does not encounter a refractory period for the CAP to progress. At a point of convergence (Figure 2), the mean output can be calculated from the sampling of phase ternary representations of the converging cone action potential as they interfere if we assign each cone activity the correct phase ternary relationship. There are many types of bipolar cell [25] and the refractory periods at convergence will almost certainly be different between types, this will give a different imprint of the total average over time, marking each bipolar cell as unique in its pattern of activity. This is reflected in the exact phase tuning of the CAP. Information from each cone receptive field is also distinct being matched to the time of distinct refractory periods and mean activity.

The cone RGC receptive fields: Cones are grouped into overlapping receptive fields [14,25,26] with about 12 cones connected directly to a bipolar cell by synapses (Figure 2) Overlapping fields allow receptive field activity to be compared and individual cone-receptive fields are mapped into the complete retina with each RGC representative of the positioning of its receptive fields. Activity from ganglion cells connecting via bipolar cells to adjacent cone-receptive fields will therefore represent multiple gradients of activity where the information from multiple-cone receptive fields forms a map of activity. As this is a direct connection it can be assumed that this information is transferred intact to the optic nerve and could be read to some extent by the central BNN. If our assumption that the output from the optic nerve to the rest of the brain is phase ternary coded is correct, this would imply that the rest of the brain also computes using phase ternary coding.

The distribution of cones and cone types within the retina ensures that shifts of external activity are registered between the cone receptive fields as light changes. This may be deconstructed to provide data expressing continuous activity gradients across the entire retina. Assimilation and coding of gradients permits shapes to be coded by comparison to other activity on the retina. By sampling and averaging from a number of cones across a receptive field the bipolar cells are able to provide information according to the reactivity of the cones and are collectively able to compute across other receptive fields.

Gross retinal coding: Using knowledge of how individual neurons function, and given the diversity of cones and their connections, it is possible to use the known connectivity and responses of neurons to elucidate and predict the functions of the rest of the retinal network and thus produce exact computational coding. Parallel computation occurs in a network when distinct inputs into the network produce distinct outputs, which are representative of the inputs.

In figure 4, we present a collection of simplified Feynman type diagrams [35] which represent the passage and collisions of CAPs over two points of convergence. This type of diagram allows us to illustrate multiple collisions and relationships over time. Time travels from left to right with computation points marked. Time has been simplified so that each phase is the same time and is equal to the refractory period at each computational step. The diagram illustrates that unique inputs into the system produce unique outputs with unique patterns of activity produced: a simple example of PTM.

The structure and coding within the retina

Assuming that the mathematical relationship is the same between the computation of the first connection, cones-bipolar cells and the rest of the retina, the synchronization and coding of the retina can be easily elicited and conclusions of what is being coded can be drawn. Using the network connections from Tsukamoto and Omi [23] frequency activity can be plotted on a diagram (Figure 5). Furthermore, knowing the resultant output and theory of the parallel coding will eventually provide an opportunity to examine the parallel data streams as they pass to the LGN, through the optic chasm (OC) and on to the visual cortex [36].

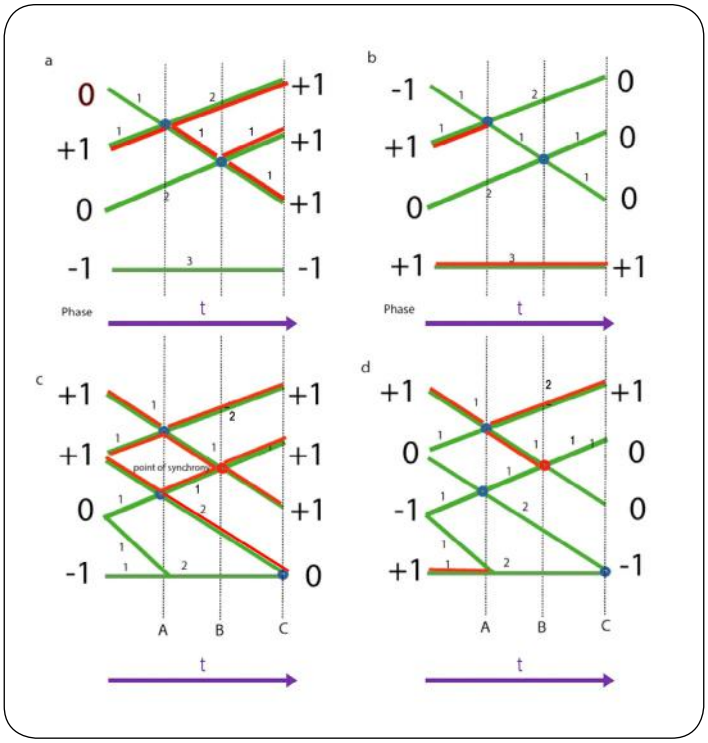

Figure 4: Passage and collisions of CAPs over two points of convergence. Numbers 0 +1 and -1 represent the initial ternary states, resting threshold and refractory of the computation action potential CAP [3]. The network is simplified such that over each connective distance each CAP does not change phase: Timing or latency between points of convergence and are written beside the network. The timing for the latency of the CAP at each point of synchronization is set to whole phase to simplify the illustration; fractional latencies will produce similar computation conformity. For the illustration latency is represented as marked in small type and is simplified to 1 phase between points of convergence. The numbers on the left represent the CAP split into its three ternary phases. The resultant value for any combination at convergence is the grouping then summation of the ternary numbers. In the retina where 12 cones converge onto a bipolar cell phases are grouped before addition indicating the resultant phase at that point. t = time. This does not depend upon neurons spiking but only upon the existence of a threshold and of a refractory period at point of convergence. The components for both exist in bipolar cells and the existence of a spike is not relevant. Phase ternary predicts that there is a point of threshold and refractory period from the mathematics of mean sampling to produce an explanation of the output of the light receptors to the retinal ganglion cells.

The maths of phase ternary convergence is to group the ternary numbers and then correct for a refractory

-1. Presence of the refractory always results in -1 except where the result is no preceding +1. A refractory -1 in any group of phase ternary competing CAP resolves to -1.

+1 (+) -1 (+) -1 = -1 = refractory,
+1 (+) +1 (+) -1 = -1 = refractory
0 (+) 0 (+) 1= 1 = threshold,
-1 (+) -1 (+) -1 = -1 = refractory.

a.) Inputs all have defined phases. The top two inputs 0 and +1 converge producing activity shown in red. At each point of convergence phase is either changed or not. c.) Demonstrates synchronicity within the network when parallel CAPs converge in a complex system stabilising and correcting error. If either top or bottom pulse is desynchronised a correction is made as in Figure 3c where a point of synchrony is indicated. If there is spontaneous activity it will be redacted. Every CAP internal to this network is similarly corrected for phase by temporal realignment to attain synchronicity over the whole network this synchronisation redacts error. b. Alternative patterns demonstrating distinct computation. c) Demonstrates a point of synchrony and d) is another computation.

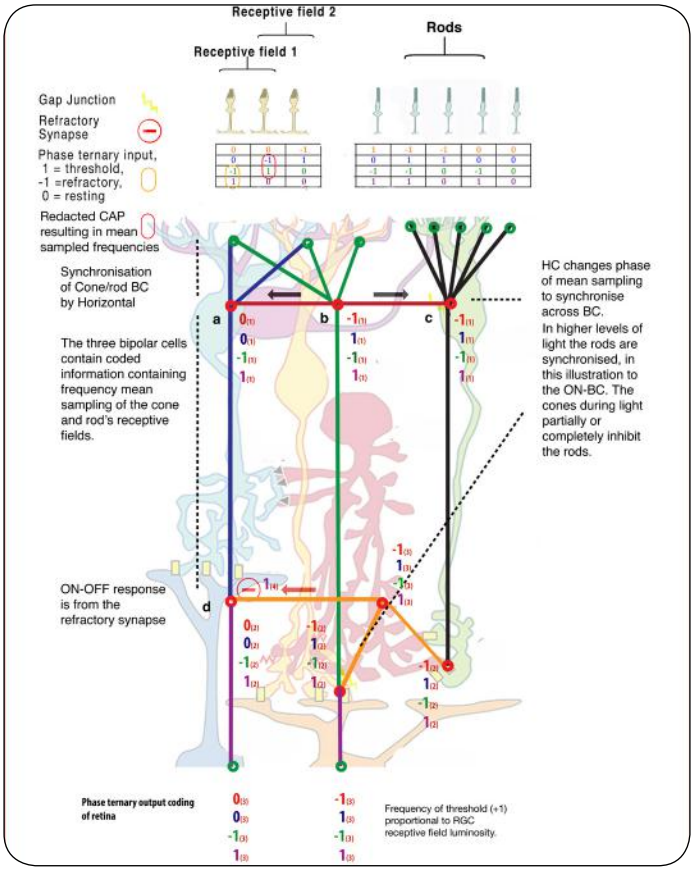

Figure 5: Representation of the retina superimposed on figure 2 as a phase ternary connective network. (Modified from Tsukamoto and Omi [21] under the Creative Commons License (CC BY)). This is same type of diagram in figure 4. Activity starts at the top of the diagram and moves along the network in any subsequently connected direction with interaction occurring at convergences. Timing is by consecutive and synchronised between points of convergence, in a real retina the timing of the CAP are independent and progress according to the speed of the axon and latency of the synapses. For the illustration latency is represented as a zero phase shift, in reality there might be two or more phase shifts for example where there are slow synapses and neurons. For this example the threshold refractory periods and the resting period are equal and equivalent to each phase. The length (a) to (b) represents 0 shift in the model - should the latency of this neuron be any fractional or complete phase it will make no difference to the demonstration of phase ternary computation. The diagram shows points of convergence and represents the phase collisions taking place with the resultant coded phase changes reflected in the output.

The box at the top represents action potential split into the three ternary phases, the four rows represent four successive phases starting at the bottom of the row and the numbers represent their respective phase.

Coloured numbers represent the number of phase changes from each coloured row from the initial. Numbers in brackets represent the computational moves from the initial cone activity. At point (a), in the first row (Purple), two cones converge to a bipolar cell their values are +1+0=+1. This result is written below each node in the network with the order of computation in brackets:1(1).

The resultant value for any combination of phase ternary is grouping then addition of the ternary phases turning all results to -1 where present. In the retina where 12 cones converge onto a bipolar cell phases are grouped before addition indicating the resultant phase at that point.

Notable points in the network: This is a synchronised system where the precision of resultant phase change is dependent upon the precision of the threshold and its ability to change the membrane to refractory.

Point (b) represents the input into the horizontal cell. Each time this neuron fires all connected neurons fire, this will synchronise the output of the cones to each

other and to the rods.

At point (c) phase input from the cones (-1 -1 0) resolves to 0. The continuous feed from the cones synchronises the rod output by changing phase originating from the cones and passing along the HC. This will synchronise the sampling initiation times of the cones and rods over three phases and permit precise error redaction and phase tuning as pulses pass to the RGC.

At point (d) is a refractory synapse -1 that changes +1 to 0. This synapse may not produce an action potential only a refractory period, for illustration we have added this to our model although it is not consequential. Point (c) both inhibits in cone reactive light and in darkness when the rods are active but not the cones. The rods therefore inhibit the resting frequency of the OFF BC when cones are inactive. Point (e) is the convergence of the amacrine cell.

Differences in synapses and the length of neurons will change the coding uniquely but will contain the same information. The exact nature of each element can be accounted for in this model.

Horizontal cells: (HCs) synapse with both cones and rods directly and through triads (Figure 2). HCs are often electrically coupled by gap junctions [31]. In PTM the ON-OFF response [23,37] is dependent upon the phase annulment of succeeding CAP. Activation of a horizontal cell by a cone cell will reset all bipolar cells within its distal connections to the same phase. This synchronization between bipolar cells permits error redaction as described above but more importantly permits phase synchronization between bipolar cells. Any change in synchronicity between ON and OFF bipolar cells due to horizontal cell reduction will therefore lead to a sporadic ON-OFF response. This effect is demonstrated by Chaya et al. [38] where the receptive field was changed when horizontal cells were removed. The horizontal cells therefore synchronize the sampling of mean luminosity over the distance of its connections.

Amacrine cells: Amacrine cells (Figure 2) synchronize and correct for error [39]. They also connect the On-Off basal ganglion permitting phase ternary computation (Figure 5).

What is encoded into these parallel information streams and how?

As streams of parallel phase ternary CAPs successively converge, collide and compute, phase changes will occur across the output of RGC reflecting minute changes in frequency from the cones/rods. These synchronized phase changes represent gradients of information relating to hue and colour and changing as the light to the retina changes.

Each RGC contains direct information not just about the direct link to the receptive field to which it is closest, but also all other connected cone receptive fields as they interact to complete the image with other types of receptor. Contextually each RGC contains the information on changing luminosity from its directly connected bipolar cell. This is the primary phase determinant, which can be modified in phase by all the other cells connected to that RGC. As convergence continues on the membrane of the RGC, phase changes are modified and corrected by further connectivity as the number of parallel connections increases. The resulting activity in figure 5 is repeated simultaneously across all synchronized RGCs producing coding by tuning the phase of the RGC at the point of parallel temporal computation. This synchronicity produces relative timing over distinct regions of the retina [21,40] and the premotor cortex [41]. It is this synchronization that permits phase ternary CAPs to redact error and function without absolute timing. Individual bipolar cells in the connected retina therefore contain the primary information of their own direct connected bipolar cells but then have convergences with other bipolar cells where information is progressively added by tuning with the result that each layer represents that above. This further integration of activity codes the geometric relationship of each light receptive field of all types into the output to the optic nerve. Thus, at the level of the RGCs the relevant information relating all connected bipolar cells to each other is encoded.

The network is lossless. Error and noise are redacted at each consecutive convergence. No information is lost between the convergence of the cones and the bipolar cells. Adjacent cone receptive fields whatever their sensitivity to hue, position or luminosity will be reflected in the combined output of the RGC in the form of overlapping gradients from the receptive fields.

Conclusions

In this paper we have described a novel type of computation, PTC, with evidence that it is the fundamental computational method used by the retina and by association the rest of the brain. PTC is computation by synchronization of action potentials in a ternary system where, in quantum computational terms, qutrits would be used as default information carriers, rather than the

qubits used in binary systems [42].

Using PTM, we are confident that there is a clear relationship between the CAP/phase ternary association and meaningful retinal computation. In this model, computation takes place without interruption at the convergences of neurons.

Given that the intrinsic morphology and physiology of neuron membranes are similar, it is logical to assume that all neurons act as phase ternary computational components, implying that PTM is the primary form of computation within physiological BNNs.

The computation of luminosity of multiple cones synapsing on a bipolar cell is performed by PTM at the point of convergence of CAPs and not at the synapses. Redaction by the refractory periods of converging CAPs eliminates all but the leading impulse resulting in sampling and averaging. The physiology of synapses defines their action in PTC as latency changers by extension of the refractory period.

The discovery of the phase-ternary CAP may signify a paradigm shift in our understanding of how neurons transmit information and also provides us with an alternative computation theory that is empirically quantifiable in all neurons from invertebrates to humans.

Declarations

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None required

Consent for publication

Both authors consent to publication.

Availability of data and material

All contained within the cited references.

Competing interests

None

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Authors' contributions

The original idea came from ASJ. Writing and construction of the article was shared 50/50 between the authors.

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5.4 Johnson AS and Winlow W (2021) Does the Brain Function as a Quantum Phase Computer Using Phase Ternary Computation? Front. Physiol. 12:572041. Doi: 10.3389/fphys.2021.572041. 1200 views.

Here I provide evidence that the fundamental basis of nervous communication is derived from a pressure pulse/soliton capable of computation with sufficient temporal precision to overcome any processing errors. Signalling and computing within the nervous system are complex and different phenomena. Action potentials are plastic, and this makes the action potential peak an inappropriate fixed point for neural computation, but the action potential threshold is suitable for this purpose. Furthermore, neural models timed by spiking neurons operate below the rate necessary to overcome processing error. Using retinal processing as our example, we demonstrate that the contemporary theory of nerve conduction based on cable theory is inappropriate to account for the short computational time necessary for the full functioning of the retina and by implication the rest of the brain.

Moreover, cable theory cannot be instrumental in the propagation of the action potential because at the activation-threshold there is insufficient charge at the activation site for successive ion channels to be electrostatically opened. Deconstruction of the brain neural network suggests that it is a member of a group of Quantum phase computers of which the Turing machine is the simplest: the brain is another based upon phase ternary computation. However, attempts to use Turing based mechanisms cannot resolve the coding of the retina or the computation of intelligence, as the technology of Turing based computers is fundamentally different.

Thus, I demonstrated that that coding in the brain neural network is quantum based, where the quanta have a temporal variable and a phase-base variable enabling phase ternary computation as previously demonstrated in the retina.

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REVIEW
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Does the Brain Function as a Quantum Phase Computer Using Phase Ternary Computation?

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Here we provide evidence that the fundamental basis of nervous communication is derived from a pressure pulse/soliton capable of computation with sufficient temporal precision to overcome any processing errors. Signalling and computing within the nervous system are complex and different phenomena. Action potentials are plastic and this makes the action potential peak an inappropriate fixed point for neural computation, but the action potential threshold is suitable for this purpose. Furthermore, neural models timed by spiking neurons operate below the rate necessary to overcome processing error. Using retinal processing as our example, we demonstrate that the contemporary theory of nerve conduction based on cable theory is inappropriate to account for the short computational time necessary for the full functioning of the retina and by implication the rest of the brain. Moreover, cable theory cannot be instrumental in the propagation of the action potential because at the activation-threshold there is insufficient charge at the activation site for successive ion channels to be electrostatically opened. Deconstruction of the brain neural network suggests that it is a member of a group of Quantum phase computers of which the Turing machine is the simplest: the brain is another based upon phase ternary computation. However, attempts to use Turing based mechanisms cannot resolve the coding of the retina or the computation of intelligence, as the technology of Turing based computers is fundamentally different. We demonstrate that that coding in the brain neural network is quantum based, where the quanta have a temporal variable and a phase-base variable enabling phase ternary computation as previously demonstrated in the retina.

Keywords: plasticity, action potential, timing, error redaction, synchronization, quantum phase computation, phase ternary computation, retinal model

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INTRODUCTION

Traditionally a nerve impulse has been considered to be an electrochemical phenomenon with experiments dating back 250 years to Galvani (Bresadola, 1998). However, this assumption has prevented contemporary consideration of the issues surrounding computation and assumes the temporal and communicative aspects of nervous activity can be resolved by electrical theory. In other words, much has been done to understand the biophysical mechanisms underlying action potentials, but not enough is known about neural computation, which is a separate issue.

The assumption that ionically based electrical communication within neurons is the fundamental processor of computation has inevitably led to models of both intelligence and computation being created using this technology in computing sciences and more recently in artificial intelligence (AI). Thus, contemporary models of nerve conduction rely on the original work of Hodgkin and Huxley (HH) and their excellent work on the action potential (Hodgkin and Huxley, 1952) that has led to the peak of the action potential as the temporal marker for computation and propagation of the action potential assumed by cable theory. The orthodox action potential (**Figure 1**) is comprised of a spike with a peak at about 0.2ms from its inception. It is evident from this curve that activation begins close to the resting potential. At resting potential, the sodium channel activates with little delay (Almog et al., 2018). The scale below **Figure 1** shows approximate distances along the axon indicating that no charge from the spike could affect activation as the main charge is prior to the point of initiation. In addition, the exponential rise of the Na^+ current demonstrates that activation of the exponential release of ions commences with little or no charge. i.e., at threshold. The molecular distances between ion channels far exceed the distance required to allow the level of charge (Hodgkin and Huxley, 1952) needed to activate progressive ion channels to achieve propagation. This is in agreement of our earlier study (Johnson, 2015; Johnson and Winlow, 2018a) where distances taken from patch clamp studies confirm that HH cable theory cannot account for propagation. At the time of HH inter-channel distances were unknown.

The HH equations are a set of nonlinear differential equations that approximate the electrical characteristics of excitable cells and can describe the electrical potential caused by exponential passage of ions notably Na^+ and K^+ when Na^+ enters through ion channels in the surface membrane. Later work indicates that some action potentials are also calcium dependent (Hayer, 1981). The equations describe the itemised potential changes of these ions over time. Propagation of the action potential along the membrane is assumed in HH to be directly due to charge from the ensuing spike opening proximal ion channels. Opening and closing of the ion channels must result in morphological changes to the ion channels proteins entailing force on the membrane. We have previously suggested that this model is insufficient to explain the activation of channels (Johnson and Winlow, 2018b) and that the activation actually moves ahead of the charge at a position where the charge is ineffective. In the HH model, propagation is assumed to be a result of capacitance change creating enough charge to affect the next ion channel on the membrane. We dispute that this is possible (Johnson and Winlow, 2018a,b) and have proposed an alternative theory for propagation (Johnson and Winlow, 2018b).

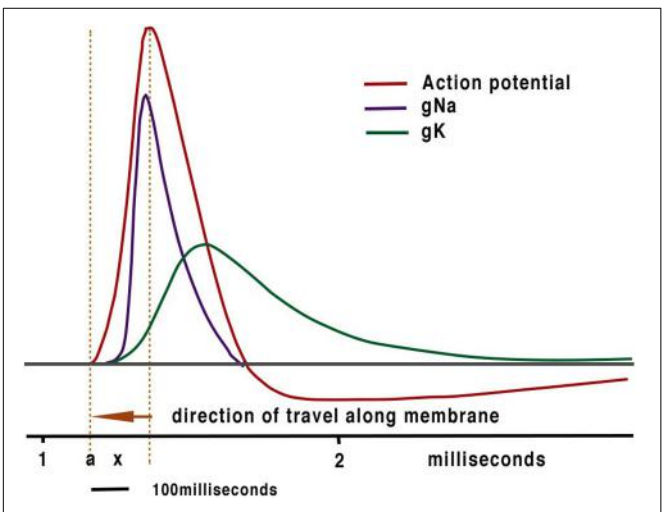

FIGURE 1 | The conventional textbook action potential showing the sodium and potassium currents with which it is associated.

ACTION POTENTIALS PLAY MORE THAN ONE ROLE IN CNS FUNCTION

We do not dispute that HH action potentials are driven by the entry and exit of ions acting down their concentration gradients, as shown in **Figure 1**, or that action potentials serve a number of functions such as:

wiring the nervous, sensory effector and neurosecretory systems during development (Pineda and Ribera, 2010);

- formation and maintenance of synaptic connections in the adult (Forehand, 2009; Dickins and Salinas, 2013; Andreae and Burrone, 2014); and
- modulation of synaptic function during and after learning (Kennedy, 2016; Rama et al., 2018).

However, it is not yet broadly accepted that action potentials are associated with underlying pressure pulses, known as solitons (Johnson and Winlow, 2018b). Taken together they form the action potential pulse (APPulse) which allows very rapid computation, as we have suggested for the retina (Johnson and Winlow, 2019). This is an important concept, given the plasticity and multiple formats of action potentials.

TIMING PLASTICITY AND ERROR

The non-uniformity of both neurons and their transmission properties is an important determinant in the type of information conveyed by them and the possible types of computation available. The brain is a large mass of neurons whose coupling, connections and form are inherently plastic. This plasticity takes various forms depending upon the timing periods sampled. Neurons may change or be replaced over weeks and months, synaptic connections may change over minutes, and conduction across synapses changes after milliseconds, ion channels within the neurons may disperse over the membrane and are regularly replaced. Many forms of plasticity affect both the temporal position of spiking neurons and their amplitude, repetitive activation inevitably changes the concentrations across the membrane which go to define the shape and timing of the conventional spike (**Figure 1**). Any change to the temporal

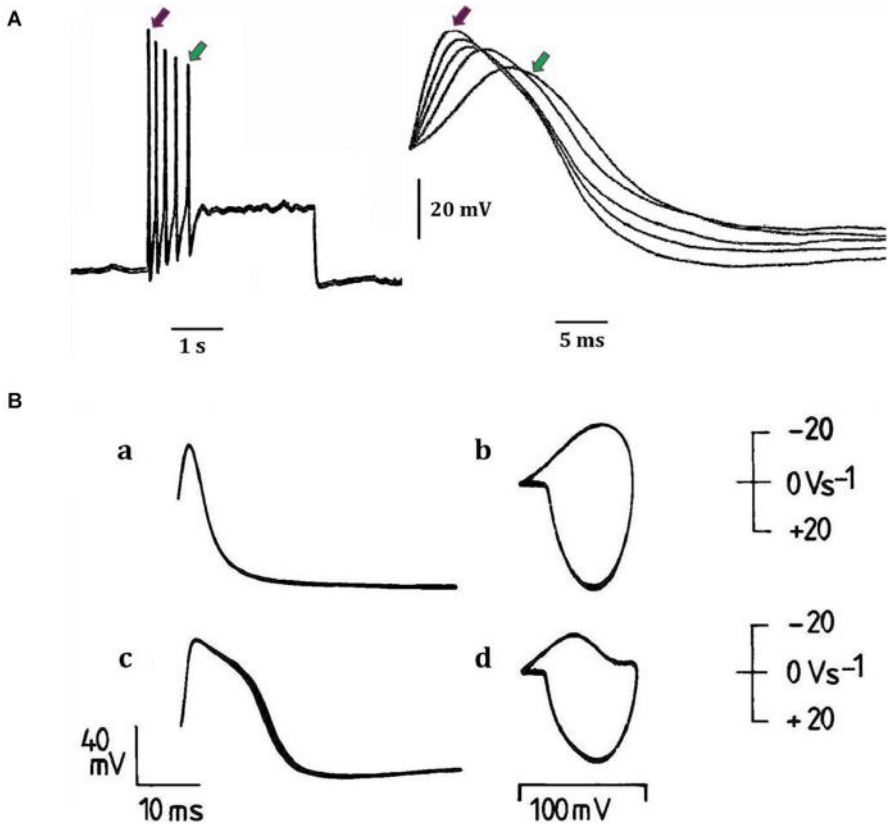

FIGURE 2 | Examples of action potential plasticity from identified neuronal somata of the great pond snail *Lymnaea stagnalis* (L.) (for locations and properties and functions (where known) of neurons and cell clusters, see Slade et al., 1981; Winlow and Polese, 2014). **(A)** Action potentials from a fast-adapting pedal I cluster cell, which was normally silent and was activated by a 3 s, 0.4 nA pulse injected into the cell via a bridge balanced recording electrode. The same five spikes are shown in each case: (a) on a slow time base, (b) on a faster time base. The red and green arrows indicate the first and last spikes respectively in each case. Note the temporal variability between spike peaks (previously unpublished data provided from William Winlow's data bank). **(B)** Different types of action potentials have different spike shapes (a and c) and trajectories as demonstrated in phase plane portraits (b and d) (a and b) demonstrate the same type 1 action potentials from an RPeF cluster neuron, while (c and d) demonstrate type 2 action potentials from an RPeB cluster neuron (adapted from Winlow et al., 1982 with permission). In the phase plane portraits, the rate of change of voltage (dV/dt) is plotted against voltage itself and the inward depolarizing phase is displayed downward, maintaining the voltage clamp convention. The technique is very useful for determining action potential thresholds (see Holden and Winlow, 1982 for details of the phase plane technique as shown here).

position of the spike peak will thus affect computation (**Figures 2**, **4**). Therefore, it is absolutely essential that for any computation to take place within a neural network this temporal plasticity must be negligible in comparison to the temporal timing. This is especially true when considering parallel processing within a network where parallel threads of information must be synchronised. For any useful consecutive computation to occur the structure must be stable, *within the relatively short timeframe of computation*, i.e., microseconds rather than milliseconds.

Action Potential Plasticity

Action potentials are thought of as the means by which cellular communication takes place within the nervous system and serve to trigger secretions from nerve terminals. They are generated by powerful ionic driving forces created by metabolic pumps such as the sodium-potassium pump, which instigate the membrane potential (**Figure 1**). However, action potentials are plastic phenomena (**Figure 2A**), whose properties vary substantially from one neuron to the next (Winlow et al., 1982; Bean, 2007; **Figure 2B**) and are often compartmentalised within neurons (**Figure 3**; Haydon and Winlow, 1982) such that the action potentials of cell bodies, dendrites, axons and nerve terminals may be quite different from one another in terms of their ionic makeup. They should be considered as a signalling mechanism for the release of secretory products at a distance from the soma (Winlow, 1989). The excellent work of Hodgkin and Huxley (1952) (HH) in determining the ionic nature of action potentials has largely obscured the accruing evidence that the plasticity of action potentials in cell bodies, nerve terminals (Winlow, 1985; Bourque, 1990; Spanswick and Logan, 1990), and axons (Rama et al., 2018) makes them unsuitable for computation within the nervous system (Winlow and Johnson, 2020). Indeed, action potential trajectories differ so much from one another that they have been used to classify different neuronal types in the neuronal somata of the pulmonate mollusc *Lymnaea*

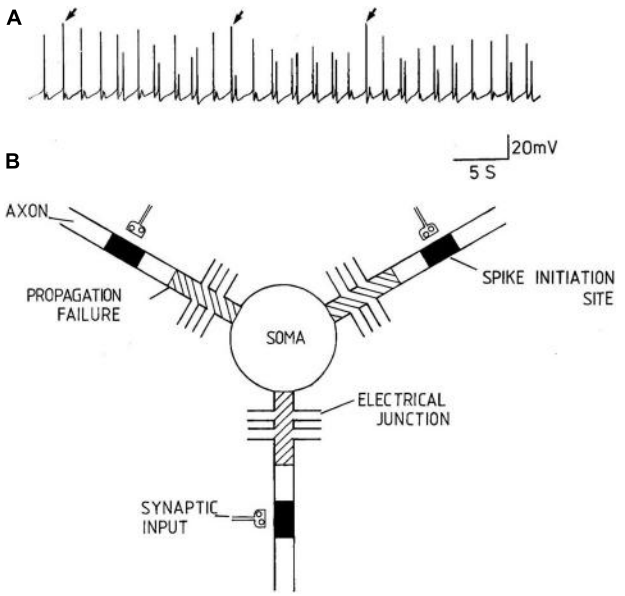

FIGURE 3 | An example of compartmentalization of axons from a multipolar pleural D group neuron of *Lymnaea stagnalis*. **(A)** Spontaneous discharges recorded from the soma of a multipolar pleural D group neuron. The discharge varies between subthreshold depolarisations of varying amplitude to large overshooting action potentials (from Winlow, 1989, with permission). **(B)** Diagrammatic model of pleural D group neurons. The filled boxes in the axons represent spike initiation sites and the cross hatching represents regions of propagation failure. Each radial axon is spontaneously active and can act to generate spikes independently of the others. The electrical synapses appear to keep the axons functionally compartmentalised from one another (Reproduced, with permission, from Haydon and Winlow, 1982).

stagnalis (Winlow et al., 1982) and in vertebrates (Bean, 2007). In particular the variable position action potential peak is well documented (Bourque, 1990; Bean, 2007; **Figure 2A**) and the maximum rates of depolarization ($\dot{V}_d$) and repolarization ($\dot{V}_r$) are highly variable phenomena and are clearly frequency dependent (**Figure 4A**) as can be demonstrated using the phase plane technique (Holden and Winlow, 1982), which is very useful for determining the threshold of action potentials (Bean, 2007; Trombin et al., 2011; Li et al., 2014; Xiao et al., 2018; Winlow and Johnson, 2020). Frequency changes result in a shift of the action potential peak and both $\dot{V}_d$ and $\dot{V}_r$ are modifiable by excitatory and inhibitory synaptic inputs (Winlow, 1985, 1987; Bourque, 1990; Bean, 2007; **Figure 4B**). In addition, neurons lie close to one another in nerve trunks and central nervous systems, so that modification of the extracellular medium by neuronal activity may alter ionic concentrations, thus modulating action potential trajectories.

Binary computational models of nervous systems usually use the peak of the spike to initiate activity (Taherkhani et al., 2020), but given the variability of $\dot{V}_d$, this is clearly an inaccurate method of computation. We have shown elsewhere that ternary phase computation is much more appropriate in modelling nervous activity where threshold is the instigator of the computational action potential (CAP): the three phases are thus: resting potential, threshold and the time-dependent refractory period, which is an analogue variable (Johnson and Winlow, 2017, 2018a,b).

SEQUENTIAL AND PARALLEL NETWORK COMPUTATION

Turing Machines

Almost all contemporary computers are designed around a Turing machine (Turing, 1937), a mathematical model of computation that defines an abstract machine, which manipulates symbols on a strip of tape according to a table of rules. The programme is provided on successive sections of the tape each synchronised externally by a clock precise to each command. Whether in using an abacus to count numbers, or a modern computer to type a scientific paper the basis of computation remains identical. At its most simple any defined set of independent inputs leading to a defined set of outputs is computation. **Figure 5A** illustrates clock timed Turing machines in binary (0,1) and in ternary notation (−1, 0, +1) (**Figure 5C**).

The philosophy of Turing compatible machines when conceived in 1936 (Copeland, 2004) was heavily influenced by not only the hardware architecture available, but also the applications to which the technology could be applied. Research into computation has always followed man-made hardware and the applications it can deliver. The establishment and acceptance of the action potential as the mechanism for nerve transmission was unavailable to Turing and his contemporaries. Unfortunately, contemporary research has largely ignored the fundamental differences between a Turing compatible machine and the brain. Recent attempts to re-imagine the brain as a Turing compatible environment are therefore a product of this process. Each step of a Turing compatible computer programme is timed by repetition of this logic by clock-steps. In parallel processing each parallel thread must also be precisely synchronised to produce logical output. Furthermore, conventional computation in neural networks, both real and artificial, relies upon selective gating of distinct routes through the network that are timed. For consistent computation to occur among parallel inputs successive parallel inputs must be synchronised precisely so that their activities can be executed.

QUANTUM PHASE COMPUTING—ANOTHER TYPE OF SYNCHRONISATION FOR THE NETWORK

Quantum phase computing occurs when temporal phase quanta, comprising base information, interact to provide a consistent output. This defines theoretical computation but does not describe the physical components needed. All modern-day computers can be visualised as quantum phase computers where the base is binary and the clock timing defines the temporal position of the quanta. In contemporary quantum computing

this takes place at the subatomic level at very low temperatures and is very fast. Nevertheless, the basic computational theory is identical to that of the nervous system as we describe below. In the nervous system quanta are in the form of quantum ternary structures (i.e., the CAP is a temporal quantum of ternary information). The CAP in the form of either the action potential or the APPulse, are quantum phase ternary structures able to interfere and synchronise within a parallel network of neurons. Synchronisation can be achieved by quantum interference in a parallel network with almost any base coding of the information. In a conventional Turing computer synchronisation occurs in binary coding because clock speed is always equal to the time taken for each phase. The levels of synchronisation are a function of the temporal precision of impulses as they collide and their format.

Quantum phase ternary computing, as in the proposed retinal model (Johnson and Winlow, 2019 and see below) is just one of an infinite number of quantum phases capable of computing depending upon phase length and base. Quanta in each case will have the form: t (base). Time t is a temporal phase variable and base can be any base. (t) is a time constant at the point of computation equivalent to relative clock speed in a Turing machine. In a Turing machine the time (t), the clock time, is the same as the phase in each computation so (t) is 1, if base is base 2. However, this is not the case in the nervous system where there is no centralised clock, because the effective clock speed (t) varies between points of convergence in a neural network as refractory periods will change at each convergence. Connected nodes in a brain neural network are therefore computing in different clock frequencies. Furthermore, in parallel processing (t) must be a consistent for each node so that during computation plasticity does not affect the network environment, as illustrated in **Figure 5D**.

Timing vs Synchronisation

Figure 5A illustrates some of the rules of parallel computation that occur within a network. The hidden neural network (?) is a combination of nodes that form into a parallel network. Parallel computation has substantial advantages over consecutive computation in terms of speed and the ability to synchronise. The values of changing parallel inputs must always reflect the synchronised changed output. The precision of synchronisation is fundamental to effective computation. For an efficient parallel network any input combination must be capable of creating a unique output and the process must be replicable. For computation to occur there must be interference between the distinct sets of inputs as they pass the nodes on the network. A pathway must be available from each input that reflects the collisions and programming within the network. In AI, a network can have programmed rules of pathways according to clock-timing. However, in the brain, clock-timing is unavailable and so another rule must exist to synchronise activity.

Figure 5A deliberately does not specify the processes or timing that must take place as indicated by the ? symbol. This implies that more than one process may take place and that further divisions of time may be present within the system. A large neural network such as the brain will have billions of connections, but to reduce error and maintain efficiency the number of components must be kept to a minimum. Synchronicity to enable successive commands in the case of a Turing machine is by a clock. This is possible because time between processes in a Turing system is not phase dependent but absolute time dependent and thus determined by clock speed. Each process in a conventional computer is therefore separated equally by time. The rule that synchronicity of processing must be centrally timed cannot apply within the central nervous system of an animal where peripheral ganglia, such as the retinal ganglia, have the ability to compute independently but must synchronise with the central nervous system. Thus, the combined output from all the neurons in the optic nerves must synchronise for us to understand the whole picture.

NEURONS IN BIOLOGICAL NEURAL NETWORKS

In contrast with an artificial neural network, a real neural network (RNN) is comprised of many neurons whose function follows their form and where neuronal morphology and function are interrelated and depend on each other (Ofer et al., 2017; Grbatinić et al., 2019). Detailed analysis of the membrane structure and function have been discussed elsewhere (Hodgkin and Huxley, 1952; Johnson and Winlow, 2018a,b, 2019). The transmission of information takes place along the membrane of the neuron and there is a finite time taken for information to pass from one point to another, this is often termed latency. A typical speed of an action potential along an unmyelinated axon in the CNS is about 0.3 m/s. From this the minimum distance between the start of the action potential and the peak of the spike can be calculated, interferometry has recorded action potential at about 30 mm/s (Ling et al., 2018; Boyle et al., 2019). Previously, Johnson and Winlow (2017) discussed how the phase ternary action potential both synchronised and corrected for error. Later we identified that the action potential exists as a phase ternary pulse (Johnson and Winlow, 2017, 2018a,b) and is defined as such by HH (Hodgkin and Huxley, 1952).

The action potential is a base 3, phase ternary structure (Johnson and Winlow, 2017, 2018a,b). The structure resembles that of a Qutrit (Xiao and Li, 2013; Almog et al., 2018) with the exception that an action potential refractory period has no effect on the resting potential – both are similarly capable of computation. We have indicated that the action potential is always accompanied by a synchronised pressure pulse (Johnson and Winlow, 2018a,b, 2019) which we refer to as a soliton. Furthermore, deformations to the membrane in the form of a pressure pulse have recently been confirmed with interferometric imaging (Ling et al., 2018; Boyle et al., 2019). We consider that for propagation to occur it is probable that this pressure pulse does not need to form a soliton only a disturbance in the membrane sufficient to open adjacent ion channels (Johnson and Winlow, 2018a). This pressure pulse is sufficient to account for conduction of information in non-spiking, spiking neurons and in hyperpolarising cells such as the cones of the retina. The

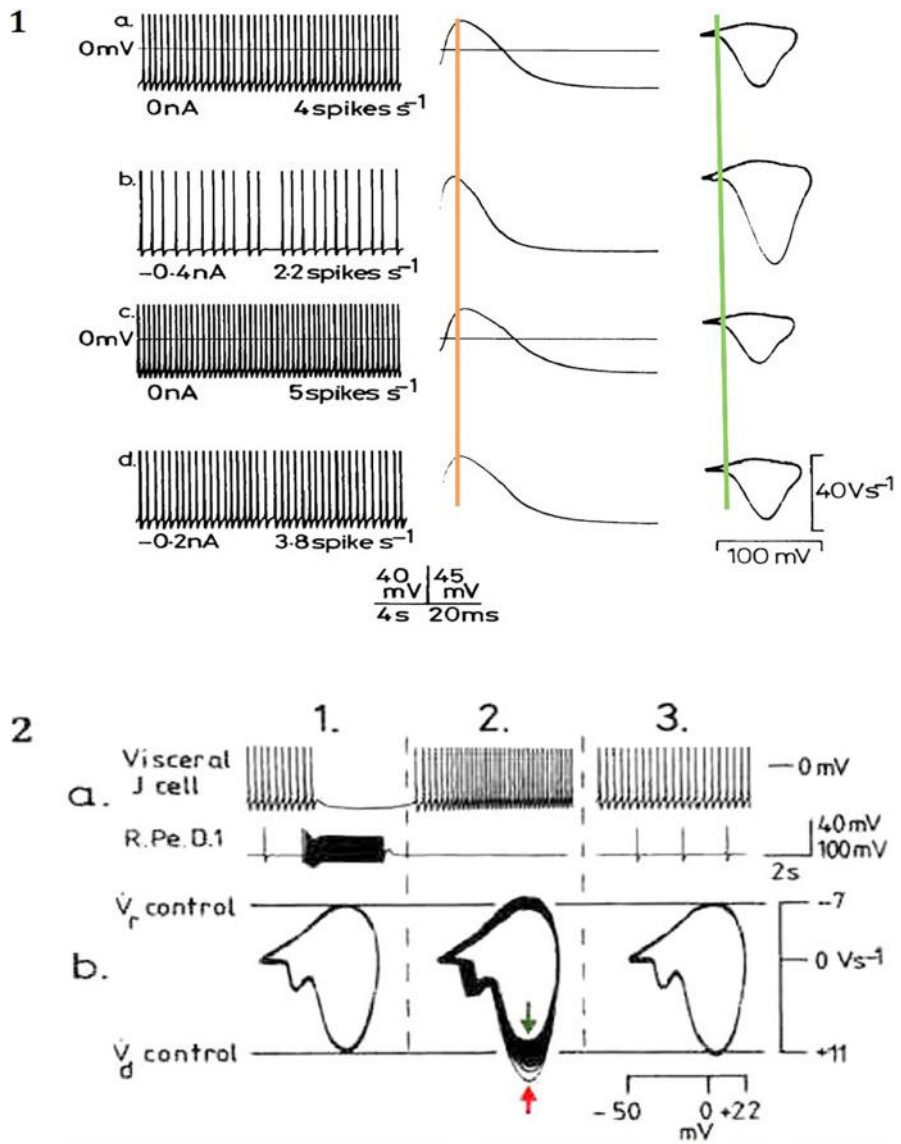

FIGURE 4 | Modulation of action potential frequency, shape and trajectories from identified *Lymnaea* neurons by **(A)** depolarising currents and **(B)** inhibitory synaptic inputs. **(A)** In a right pedal A (RPeA) cluster neuron, action potential frequency, shape and phase plane trajectory are modified by maintained depolarizing and hyperpolarizing currents, which modify the action potential properties. All spikes in the traces at left are superimposed in the middle trace and in the phase plane portraits at right. Note how the action potential peak at $\dot{V}_d$ shifts either side of the superimposed orange line, while threshold (superimposed green line) remains temporally constant and $\dot{V}_d$ is even more clearly variable in the phase plane representations. Similar effects are produced by excitatory and inhibitory synaptic inputs (adapted from Winlow et al., 1982 with permission). **(B)** Effects of monosynaptic i.p.s.p.s from the giant dopamine-containing neuron, RPeD1 (right pedal dorsal 1), on visceral J cell action potentials (for detail see Winlow and Benjamin, 1977). (a) Upper trace, J cell; lower trace RPeD1 (ac coupled and filtered). (b) Phase plane portraits of J cell action potentials: 1. pre-control; 2. phase plane portraits of 32 successive action potentials (peak of spike 1 denoted by red arrow, spike 3 by green arrow); 3. post-control of the last 10 action potentials shown above. After inhibition by RPeD1 both $\dot{V}_d$ and $\dot{V}_r$ were increased, but as the cell accelerated following inhibition both $\dot{V}_d$ and $\dot{V}_r$ declined below control values (adapted from Winlow, 1987, with permission).

temporal precision of this synchronised pulse is much greater than that of the HH action potential as its speed is determined by the structure of the membrane that has a rate of change many times slower than that of either computation or even action potential conduction. Basically, the soliton activates the ion channels that then add entropy to the pulse; the speed of the pulse is then defined by static membrane components. Temporal plasticity of membrane transmission occurs at a far slower rate than that of the ionic exchanges in HH. Temporal error is therefore minimised in the APPulse. The component structures of the APPulse were then deconstructed into computational component parts to form The Computational action potential CAP (Johnson and Winlow, 2017). The CAP is a mathematical representation of a ternary quantum pulse where, during a

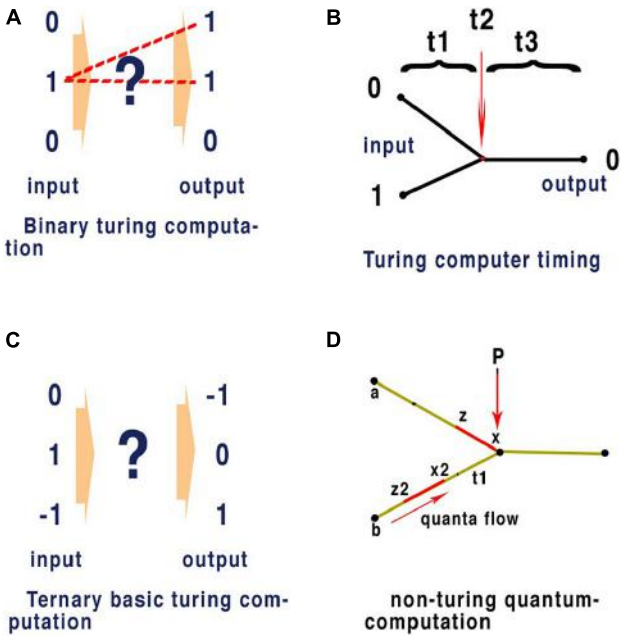

FIGURE 5 | In quantum computation network timing does not require an external clock. This form of computation only requires input to reflect consistent outputs for given values and here we compare it with Turing computation where clock speed synchronises input with output. Panel **(A)** is an illustration of conventional binary computation of inputs and outputs. The exact network that separates the input from the output is indicated by (?) and the most obvious pathways are shown as dotted lines and gating within the network (?), forms this pathway. This gating represents a single command and must be executed within the set time allocated between the input and output as defined by some external clock. (?) May represent a complex network, flow through the network is defined node to node by precise timing between those nodes in exactly the same way and the outputs are always synchronised by clock-speed (as in the central processing unit of a computer). Panel **(B)** illustrates binary computation in a conventional space split between input nodes with binary properties of (0) and (1) respectively and giving a result of (0). (t1) Represents the timing from input nodes (0) and (1) respectively. (t2) Represents the time taken for the gated node at (t2) to react to the inputs (0) and (1) according to a set program that defines the output from these specific inputs as being (0). (t3) Represents the time from the gated output to the exit node. Quantum computing applied to 5 panel **(B)** combines t1 + t2 to represent one quanta of binary information in a parallel network. In a conventional neural network computer as used in AI or SpiNNaker (Furber and Temple, 2007) an emulator using spiking AP, where latencies of timing are fixed and arranged so that (t1) and (t3) are uniform t1 + t2 +t3 are equivalent to clock speed effectively synchronising each command. This is the mechanism apparent from the definition of a Turing type machine and evident in all practical applications of modern computing. Panel **(C)** is an illustration of a ternary Turing machine, note synchronisations are performed by external clock and this is not applicable to a brain neural network. Panel **(D)** illustrates a non-Turing quantum machine such as evidenced in the brain neural network. P is a point of convergence thus computation in the network and timing between nodes a and b to P of quanta (in red) is determined by the speed at which quanta flow between the nodes (black dots). Computation at P depends upon the quantal information (which may be any base), the mechanism at P, and the timing of interference between quanta. In a binary Turing machine **(B)** the quanta are fixed between t1+t2 by clock speed. In a brain neural network timing is defined by the speed of neurons and not by a clock. For the APPulse or action potential, x to z, and x2 to z2 are represented by the threshold to refractory periods of each quantum, respectively: computation at the P is defined by the timing of each quantum and how they *(Continued)*

FIGURE 5 | Continued
react on collision. In the case of the action potential and the APPulse collision between threshold and refractory periods results in annulment of the succeeding quanta. This results in computation through the network. This is a fundamental process in quantum computation and can be applied to all base and temporal computation in a neural network where the rules at P may differ.

collision of two impulses, if a threshold crosses a refractory period the threshold is annulled. The CAP is equally valid for ternary quantum computation by either HH or the APPulse. The difference between the two lies in the temporal precision of successive impulses up to 10,000 times greater with a pressure pulse than with HH and cable theory (Johnson and Winlow, 2018b). Importantly the CAP assumes that the temporal pulse starts on activation and not from the spike peak as shown in **Figures 2A**, **4A**.

Activation of Propagation

As shown above, assuming the peak of the action potential to be the timing cue of the nerve impulse is a fundamental oversimplification of the mechanics of propagation because the underlying statistical event triggering the action potential must be the point of temporal precision for computation and synchronisation, i.e., threshold. To be effective across parallel inputs determined by phase, temporal precision is critical to phase computation. The accuracy of phase at the point of convergence must take place within a substantially reduced timeframe where temporal plasticity of membrane microstructure is close to zero.

Parallel Computation

One functional test of whether the transmission of information is performed by HH cable theory or by the APPulse is whether they are capable of computation within the known neural networks of the body. Neural networks in the brain process information between parallel inputs across disordered networks (**Figure 6**). The phase at which each CAP arrives at a point of computation is thus a quantum of ternary information. CAPs moving within a neural network compute by collision diffraction along specific pathways defined by temporal geometry forming patterns and changing outputs (Johnson and Winlow, 2018a,b).

Computation of CAPs in a neural network occurs naturally through the phenomenon of phase cancellation at each node (Johnson and Winlow, 2018b), where CAP's interfere with each other. The mathematics of these interferences is defined by the precision of the activation point of the CAP, i.e., what is assumed to be a threshold in HH. To understand this crucial element of parallel processing it is necessary to look more closely at the molecular level of the components. The effects of interference between CAPs is illustrated in **Figure 5**. This illustration is equally valid for computational precision across multiple neurons in a network where interference must temporally synchronise. In **Figure 6D** CAP's flow from left to right across the surface of the three-dimensional axon of the neuron. When the activation-threshold encounters a refractory section of membrane it is annulled.

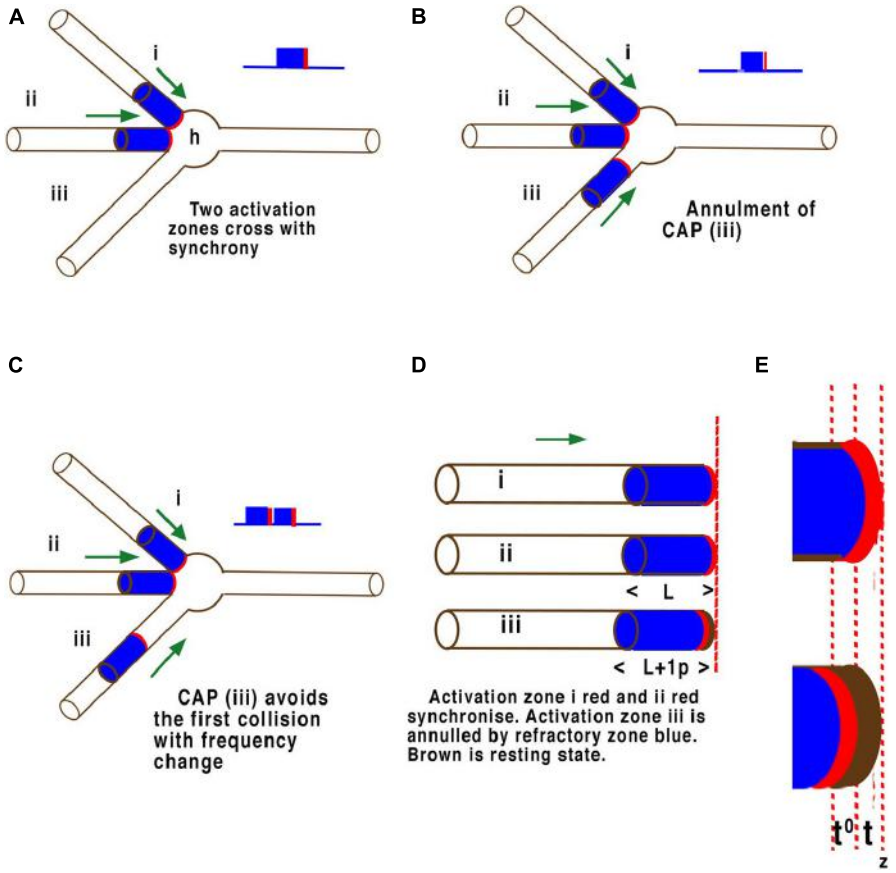

FIGURE 6 | Examples of quantum phase ternary interference between CAPs. Panels **(A–E)** are illustrations of CAP's travelling along the surfaces of the neuron membrane just before collisions at the axon hillock (h). The threshold of each CAP is highlighted in red while the refractory period is coloured blue. These are not to scale. Panel **(A)** illustrates a single neuron with three axons converging on an axon hillock or cell body. Two CAP's (i) and (ii), move in the direction of the axon hillock where they will collide. In this case both CAP's (i) and (ii), are in phase with the thresholds overlapping. These CAPs will fuse and continue a single CAP. **(B)** The same two CAP's (i) and (ii), are in phase with the thresholds overlapping and will fuse and continue as one CAP as in panel **(A)**, but a third CAP (iii) arrives slightly after the other two and its threshold encounters the refractory period of the other two and is annulled. **(C)** The same two CAP's (i) and (ii), are in phase with the thresholds overlapping. These CAP will fuse and continue as one CAP as before, but in this case the threshold of the third CAP (iii) arrives at the axon hillock after the first two have fused and their respective refractory periods have no effect on it. The result is that two CAPs will pass into the axon at right. In panel **(D)** (L) represents the refractory time of each CAP, whilst in panel **(E)** (t) represents the timing of the activation-threshold at (z), the point of computation. In a parallel network **(D)** where CAP's converge any time greater than (t) and less than (L) will result in phase change. This phase change changes the distance between successive CAP's and therefore frequency. Where activation-thresholds become desynchronised phases become annulled.

THE RETINAL MODEL OF NEURAL COMPUTATION

In a previous paper, Johnson and Winlow (2019) described the neural coding in the retina and further details and references are to be found in that paper. **Figure 7B** is a schematic diagram of the relevant central elements of the retina. Light from the right of the diagram falls on grouped light receptors GLR (large coloured ovals that can be rods or cones). These light receptors are connected through a static array of bipolar neurons to retinal ganglion cells (RGC). Light receptors have an average of 12 per group and up to 25 that connect to a single bipolar cell whose output is usually observed in the RGCs (Behrens et al., 2016). When all other connections are supressed each ganglion cell is activated by connected light receptors as shown in the diagram. Circular patterns of light receptors lie adjacent to one another or overlap each other and each is connected to a ganglion cell.

Observations From the Retina

Adjustments in light intensity results in a corresponding frequency change of action potential in the RGCs. The frequency of synaptic discharge from the cones is also similar to that of output t and t1 in **Figure 6B**. The frequency of discharges of all the light receptors attached to a bipolar cell result in a mean change to output frequency RGC (t) and (t1), respectively. Light on the retina and mean light receptor discharge frequency is therefore proportionally related to output at the RGCs. In **Figure 6B** changes in light intensity on the grouped light

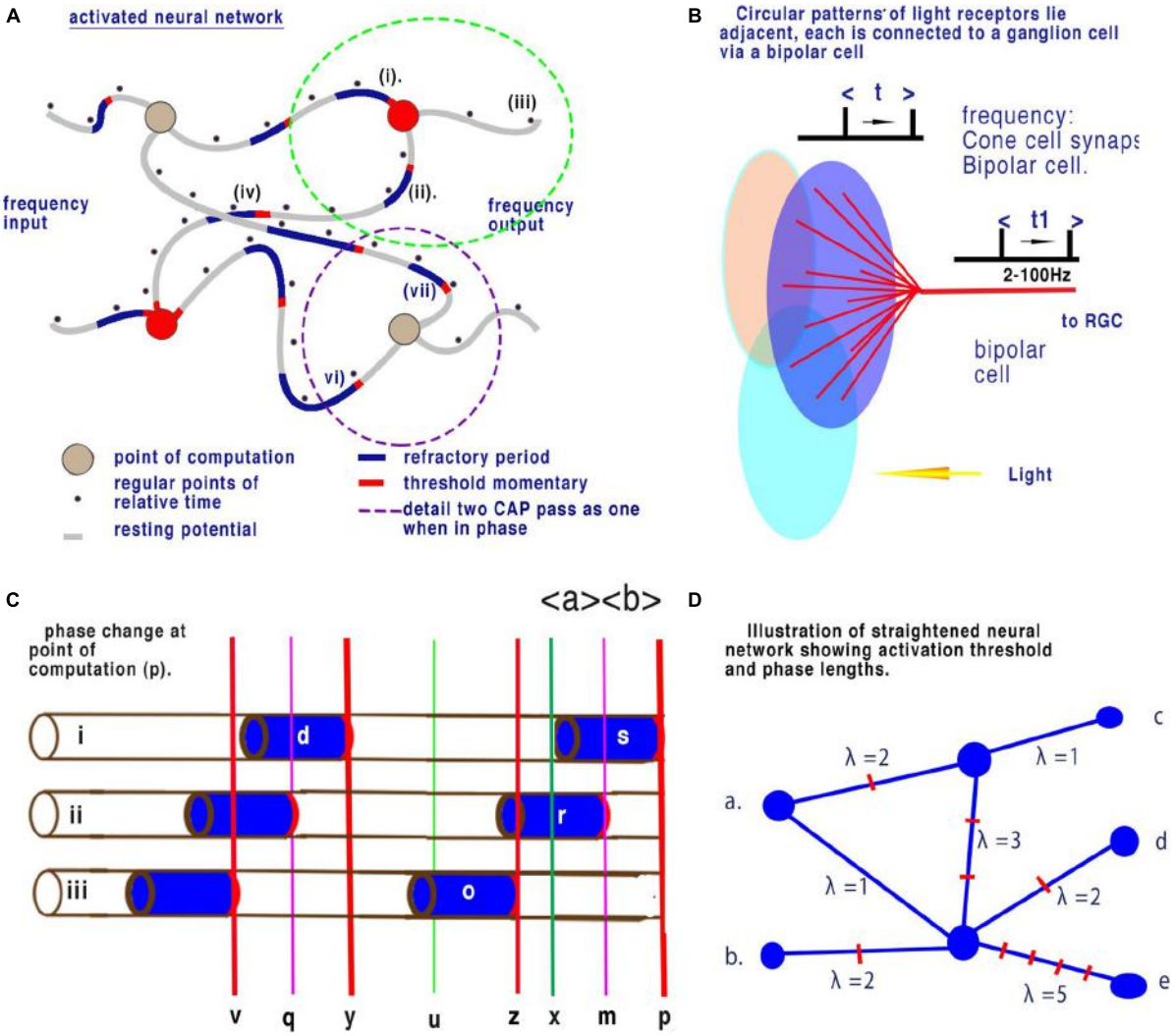

FIGURE 7 | Neural networks in the retina. **(A)** Illustration of a brain neural network showing changes in latency between nodes (from Johnson and Winlow, 2019). Panel **(B)** shows 12 cones (blue lines) converging upon a single bipolar cell (red lines). Here time, t1, changes relative to t. Panel **(C)** is an enlargement showing a succession of CAPs arriving at the point of computation (p). During fixed light intensity each cone will cause CAP's to flow towards the point of computation (p) and CAPs are moving from left to right. Activation-thresholds that allow a CAP to continue after computation are shown by the red lines. All CAP in neurite (ii) are annulled. The time (p) to (x) is equivalent to the refractory period of the CAP of neuron (i). Refractory period (s) is selective and all other CAPs are annulled during this period. The result is that time between points of computation (z) to (p) is dependent upon only the time from the end of refractory period (x) to the next activation-threshold (z). This is then reflected in the spike timing from the RGCs. The interval (x) to (z) over any time period is a mean sample when calculated between successive CAPs. This results in the calculation of mean sampling by quantum phase ternary computation. Before collision the timing of each successive CAP along a neuron can be considered as a minute change in frequency. Panel **(D)** illustrates a straightened disordered brain neural network such as in Panel **(A)**. Frequencies of inputs (a) and (b) result in changes to frequency outputs (c), (d), and (e). In the network of (d) distances have been resolved into phase lengths of quanta. The network represents computation between different time dimensions illustrated by λ. As quanta arrive at different time at each node (between nodes quanta may arrive asynchronously but are digitised) convergence and interaction will take place where phases differ. This interaction will result in distinct changes in frequencies of output for distinct changes in frequencies of input.

receptors results in a mean frequency change of action potential at the output from the bipolar cells (t1). For this computation to occur there must be a point of computation (Johnson and Winlow, 2019).

There appears to be only one mathematical mechanism that can generate the mean frequency of parallel streams of action potentials arriving at the LGN, i.e., mean sampling at the point of convergence of the bipolar cells. At the point of convergence, the refractory period of one CAP will block any succeeding CAPs for that period of time. This refractory period is dependent upon the membrane constituents and its timing is critical to computation. In a parallel system where CAPs converge when the first CAP passes through a point its refractory period blocks further action potential. When this CAP's refractory period ends there is a space in time before the next CAP's activation threshold, this is equivalent to the mean sampling of cone CAPs.

Digitisation of Light Receptor Outputs by Phase

Using the known speed of CAP in the bipolar cells and the frequency of discharge, the minimum distance between each successive discharge can be calculated. The frequency rate of discharge from the bipolar cells has been measured at 2–100 Hz. Velocity of action potentials vary from neuron to neuron, measurements for an unmyelinated axon vary from over 25 m/s in the squid to 0.3 m/s or below in brain tissue. The neural networks of the CNS and retina contain small unmyelinated neurites and the smaller figure is used in our calculations. The velocity of action potential for an unmyelinated axon has been measured by interferometry in nerve tissue at about 0.3 m/s (Boyle et al., 2019).

Calculation of Precision

The maximum frequency of the CAP is determined by the timing of the refractory period. If the maximum frequency is 100 Hz and the speed is 0.3 m/s then the distance from the threshold-activation to the end of refractory period is: 0.3/100 = 0.003 m or a time of 1 ms. This figure corresponds to observed measurements of the absolute refractory period (Purves et al., 2001). The activation mechanism for activation-threshold is likely to be timed less than 1–10 μs.

Single action potentials are not temporally accurate to less than 0.1 milliseconds measured from the spike peak, or even threshold, so computation in the eye is incompatible with the action potential, where reliability of temporal measurement is in order milliseconds. However, a pressure pulse or soliton is formed at the molecular level of the membrane and travels through the membrane at a constant speed with a greater accuracy than required (Heimburg and Jackson, 2005) for quantum phase ternary computation.

For the observed changes(Grimes et al., 2014) in frequency when light shines on the cones, CAPs must be formed and compute at the convergences of the bipolar cells and the CAP must have an activation-threshold of below 10 μs. This timing is critical when we consider attempts to mirror nervous communication using models of spiking neurons obeying HH cable theory. In parallel processing the temporal precision is critical to operation. In a conventional view of nerves where computation takes place at the synapses.

Information Contained Between Nodes

Figure 5B illustrates a conventional binary neural network. During time, as defined by clock speed, one bit (0 or 1) is connected to the output. In a neural network this means that node to node contains one bit. **Figure 7C** illustrates quantum ternary phase pulses represented by CAP. If each refractory period is separated digitally into 12 (as above) then CAP (s) can be subdivided into 12 positions of phase change. In **Figure 7A**, CAP (r) is annulled because its activation-threshold at point (m) will cross the refractory of CAP (s). The position of (m) is critical to computation because phases from (x) to (z) have been redacted. Each of the 12 subdivisions of the refractory period therefore code for one trit of information. In conventional computing terms the effective clock speed of computation for the bipolar cells is about 10 μs during which time each neuron connected to a convergence conveys 1 trit of information base 3. The space along a neuron of two impulses conveys base 9 information. The information contained is therefore much greater than possible with binary. There are many areas of the nervous system where connections similar to that of the bipolar cells are apparent for example the bipolar connections of the auditory system(Petitpré et al., 2018).

QUANTUM PHASE TERNARY COMPUTATION IN A NETWORK

Figure 7D illustrates a straightened disordered brain neural network such as **Figure 6A**. Frequencies of inputs (a) and (b) result in changes to frequency outputs (c), (d), and (e) as described in **Figure 7C**. The nodes are the convergences. In **Figure 7C** distances between all nodes have been annotated with the phase length of the frequency λ of CAP showing different distances. The phase length is the distance between two CAP activation thresholds (analogous to spike timing). Between any two nodes this phase length will remain constant during minimum plasticity, as it is dependent upon the integrity of the membrane. Between (a) and (c) λ = 3 so there are 3 CAP. Between (a) and (e) λ = 6. Time for CAP between (a) and (c) is half that of (b) to (e). If the frequency of (a) changes from 1 to 2 the frequency of (a) to (c) changes 3 to 6 while frequency of (a) to (e) changes 6 to 12 or a change of 3 or 6 Hz depending upon the route. Interference between quanta in this system annul and change the direction of CAP within this network. As the frequency changes at inputs (a) or (b), output frequencies at (c), (d), and (e) change accordingly such that they provide a unique reference to the frequency inputs.

CONCLUSION

- In neural computation the action potential peak is unreliable for calculations because of action potential plasticity. Threshold is much more clearly temporally defined.
- The frequency of action potentials at the retinal ganglion is a result of interference between action potentials temporally measured only from the point of activation-threshold eliminating peak action potential-timed computation. In addition, the temporal accuracy of threshold activation must be less than 10 μs indicating that the Hodgkin Huxley action potential alone is incapable of computation.
- At the set point on the membrane where activation takes place charge from the spike is minimal. Thus, activation is the cause of the action potential and must be responsible for adjacent further activation.
- Computation during plasticity results in error in a neural network, which must be redacted, and we have proposed a method of phase ternary redaction. Spike timed computation is untenable because the spike arrives after the activation-threshold and the spike is a plastic phenomenon.

- The implication in terms of computer science is that in a parallel neural network, Turing based machines are a small subset of Quantum Phase Computing.
- Computation within brain neural networks is most likely by quantum phase ternary process, distinct from and much more precise than the action potential.

AUTHOR CONTRIBUTIONS

AJ came up with the original idea quantum phase computing in the brain to which WW added information on action potential plasticity. Both authors worked together to produce the final document.

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5.5 William Winlow and Andrew S. Johnson. Nerve Impulses Have Three Interdependent Functions: Communication, Modulation, and Computation. Bioelectricity.Sep 2021.161-170.http://doi.org/10.1089/bioe.2021.0001

Comprehending the nature of action potentials is fundamental to our understanding of the functioning of nervous systems in general. Here I considered their evolution and describe their functions of communication, modulation, and computation within nervous systems. The ionic mechanisms underlying action potentials in the squid giant axon were first described by Hodgkin and Huxley in 1952 and their findings have formed our orthodox view of how the physiological action potential functions. However, substantial evidence has now accumulated to show that the action potential is accompanied by a synchronized coupled soliton pressure pulse in the cell membrane, the action potential pulse (APPulse). Here I explored the interactions between the soliton and the ionic mechanisms known to be associated with the action potential. Computational models of the action potential usually describe it as a binary event, but we suggest that it is a quantum ternary event known as the computational action potential (CAP), whose temporal fixed point is threshold, rather than the rather plastic action potential peak used in other models. The CAP accompanies the APPulse and the physiological action potential. Therefore, I conclude that nerve impulses appear to be an ensemble of three inseparable, interdependent, concurrent states: the physiological action potential, APPulse, and CAP.

There have been many discussions and correspondence with many researchers on this subject and the impact.

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Perspective

Nerve Impulses Have Three Interdependent Functions: Communication, Modulation, and Computation

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Abstract

Comprehending the nature of action potentials is fundamental to our understanding of the functioning of nervous systems in general. Here we consider their evolution and describe their functions of communication, modulation, and computation within nervous systems. The ionic mechanisms underlying action potentials in the squid giant axon were first described by Hodgkin and Huxley in 1952 and their findings have formed our orthodox view of how the physiological action potential functions. However, substantial evidence has now accumulated to show that the action potential is accompanied by a synchronized coupled soliton pressure pulse in the cell membrane, the action potential pulse (APPulse). Here we explore the interactions between the soliton and the ionic mechanisms known to be associated with the action potential. Computational models of the action potential usually describe it as a binary event, but we suggest that it is a quantum ternary event known as the computational action potential (CAP), whose temporal fixed point is threshold, rather than the rather plastic action potential peak used in other models. The CAP accompanies the APPulse and the physiological action potential. Therefore, we conclude that nerve impulses appear to be an ensemble of three inseparable, interdependent, concurrent states: the physiological action potential, APPulse, and CAP.

Keywords: nerve impulse, physiological action potential, soliton, action potential pulse computational action potential

> ''The great sins of the world take place in the brain, but it is in the brain that everything takes place. it is in the brain that the poppy is red, that the apple is odorous, that the skylark sings'' (Wilde, 1891[1]) and the soliton and the action potential are the primary elements underlying sentience[2]—they need to be better understood.

Introduction

Traditionally a nerve impulse has been considered to be an electrochemical phenomenon with experiments dating back 250 years to Galvani.[3,4] However, this assumption has prevented contemporary consideration of the issues surrounding computation and assumes the temporal and communicative aspects of nervous activity can be resolved by electrical theory. In other words, much has been done to understand the biophysical mechanisms underlying nerve action potentials, as a result of the excellent work by Hodgkin and Huxley[5] and their successors. However, substantial findings that a soliton wave accompanies the physiological action potential[6] is not yet widely accepted, nor are suggestions that a computational form of the action potential[6] may also exist. Although the Hodgkin-Huxley model describes the ionic movements that lead to changes in electrical potentials across nerve cell membranes, which lead to action potentials, it cannot by itself provide the temporal accuracy of computation. However, when combined with the soliton to form the action potential pulse[2] (APPulse), the ionic movements associated with the action potential may be seen as part of a process that provides entropy to the soliton. This then defines the speed of the pulse that determines the temporal characteristics that define the computational characteristics of each neuron. Here we explore these concepts in more detail and consider whether they are interrelated manifestations of the nerve impulse. To set the scene we start by considering the evolution of action potentials and then their functions within nervous systems.

Evolution of action potentials

If we are to understand the nature of neuronal action potentials, we first need to understand how they might have evolved. However, although action potentials occur in many

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neurons, they are not by themselves a diagnostic feature of neurons as we will see below because they also occur in a wide variety of tissues and organisms, including plant cells and bacteria.

Phylogenetic considerations. In the nerveless placozoa, the simplest known free-living animals, inducible fast sodium action potentials have been demonstrated,[7] as have Ca_v3-like channels. This demonstrates that electrical signaling occurs ubiquitously in cells other than neurons or muscle, including unicellular organisms,[8] plants[9] and may also occur in some human carcinomas where electrical activity appears to be driven by voltage-gated sodium channels.[10,11] Electrical signaling also occurs in bacterial biofilm communities through release of intracellular potassium[12,13] that depolarizes nearby cells via voltage gated potassium channels (K_V)[14] and coordinates metabolic states among the cells.[12] This depolarizing wave occurs in the absence of voltage-gated sodium (Na_v) or calcium channels (Ca_v), which are commonly found in bilaterian nervous systems[15] alongside K_v channels. Additionally, action potentials have also evolved in fungi and plant cells,[9] but are much slower than those of the squid giant axon, by a factor of 10^3 and depolarization is driven by efflux of chloride ions and influx of Ca^{2+}, which opens the Cl^- channels, rather than influx of Na^+.[15] Repolarization appears to be driven by increased permeability to potassium and protons.[16,17]

Which membrane channels came first? Kristan[15] speculates that K_v channels, used to regulate cell volume in response to membrane stretch, were the only voltage-gated channels in the earliest animals, followed by Ca_v channels to control metabolic state, regulate cilia,[8] and muscle contractions. However, given that calcium is highly toxic to cytoplasm, calcium channels may have been the first to evolve alongside appropriately linked metabolic pumps for calcium removal. A combination of these channels could generate relatively slow action potentials in small soft-bodied animals at the dawn of metazoan evolution, so what drove the addition of Na_v channels? Was it the need for more rapid movements as predation of one species on another became prevalent? We will never know, but whatever was the original selective advantage, shorter duration action potentials evolved, enhanced by the probable parallel evolution of fast K_v channels to rapidly terminate them. Furthermore, Kristan[15] also provides evidence that neurons may have evolved more than once by parallel evolution, most usually from epidermal cells,[18] but in some cases from endodermal cells, for example, in cnidaria,[19] as also appears to be true in gastropod molluscs such as *Aplysia*.[20] This suggests that wherever the appropriate genetic machinery is available to generate the appropriate channel proteins, action potentials may result. This appears to be the case in several human carcinoma models, where voltage gated sodium channels[10,11,21] and calcium channels[22] have been described and may be involved in action potential generation.

From an evolutionary standpoint it might be best to consider neurons as stretched secretory cells,[20,23] sometimes modified to communicate over long distances by means of fast action potentials, as in the case of alpha motor neurons. In other cases, the cells remain short and transmit by graded transmission, as in the case of the spikeless neurons of the retina or the olfactory bulb in vertebrates[24,25] and many other examples in invertebrates.[26] Thus, there is no such thing as an orthodox neuron: they have hugely variable morphologies and nerve cell membranes vary in their properties, depending on their location and functions. However, once the appropriate genetic machinery evolved, action potentials became possible and are utilized where necessary in nervous systems where they are known to serve several functions.

Neuronal action potentials have multiple functions

Action potentials in neurons serve a number of functions in the nervous system, which may be summarized into the following three areas:

- *Communication*:
  - wiring the nervous, sensory effector and neurosecretory systems during development[27]
  - transmission within the adult nervous system, including formation and maintenance of synaptic connections[28–30]
- *Modulation* of synaptic function and memory storage after learning[31,32]
- *Computation* within neurons and brain neural networks.[2,33,34]

Communication Within Nervous Systems

Action potentials can be conducted quite rapidly in invertebrate and vertebrate myelinated axons[35] (up to about 120 m/s in alpha motor neurons) and large diameter invertebrate fibers such as the squid giant axons (ca. 25 m/s), each of which are in essence a fusion of the axons of two interneurons.[36] However, this does not appear to be rapid enough for the process of neurocomputation (see section on Computation Within Nervous Systems). In addition, there is a timing problem when the action potential peak is used as a time point as in many computational models (e.g., Taherkhani et al.[37]). This is because action potentials are plastic phenomena[38,39] and the timing of the action potential peak varies considerably with spike frequency making it unsuitable for computational modeling and coding in the brain. This is demonstrated in Figure 1, where successive action potential peaks vary temporally from one another (Fig. 1B). However, the threshold point of initiation (Fig1C) remains constant. Thus, threshold is a more appropriate temporal fixed point for neural communication and subsequent computation.[34,40] In nonspiking neurons, the threshold will be the point at which voltage-gated channels open to instigate transmitter release.

There is accumulating evidence of both electrical and mechanical components in action potential propagation

One of the key points in our understanding of the functioning of nervous systems in general was the discovery of the ionic mechanisms underlying the membrane potential and the action potential, powered by ionic pumping mechanisms. However, substantial, and overwhelming, evidence continues to accumulate to show that this is not the whole story, because the action potential is always accompanied

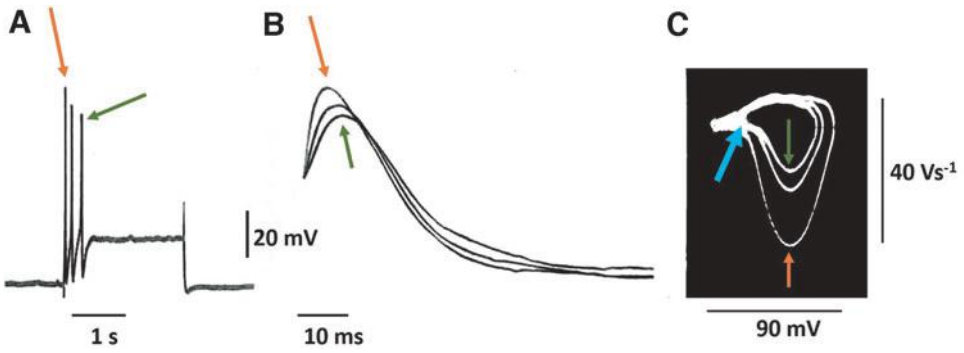

FIG. 1. Plasticity of action potential shape and action potential peak recorded from the soma of a fast-adapting pedal I cluster neuron in the intact brain of the mollusc *Lymnaea stagnalis* (L.). The cell was normally silent and activity was initiated by a 0.2 nA current pulse of 3 s duration injected into the cell via a bridge balanced recording electrode. The same three spikes are represented in each case; **(A)** on a slow time base, **(B)** on a faster time base, and **(C)** as a phase plane portrait in which rate of change of voltage (dV/dt)is plotted against voltage itself and the inward depolarizing phase is displayed downward maintaining the voltage clamp convention. In each trace the peak of the first action potential is indicated by an *orange arrow*, the second action potential peak is unlabeled, and the third action potential peak is indicated by a *green arrow*. The three successive spike peaks clearly vary temporally from one another, but the threshold point of initiation remains constant as indicated in **(C)** by the *blue arrow* in the phase plane portrait (from Winlow and Johnson,[40] licensed under Creative Commons BY-NC-SA 4.0).

by a synchronized coupled soliton pressure pulse in the neuronal cell membrane[2,33] (Table 1), which taken together form the APPulse[6] that instigates channel opening (Fig. 2).

Rather than detracting from the Hodgkin and Huxley (HH) model, the biomechanical data should be seen to enhance it and should not be ignored. Unless either component can be shown to be both necessary and sufficient to generate the action potential, we should assume that that membrane biophysics and biomechanics are inextricably linked to generate the APPulse. In essence, Hodgkin and Huxley[5] demonstrated that sodium ions entering an axon generated the rise of the action potential and that this was counteracted by the opening

Table 1. The Action Potential Pulse—There Is Accumulating Evidence for the Soliton Pressure Pulse in Neuronal Membranes

- A ''soliton'' mechanical pulse accompanies an action potential and is stable propagating at constant velocity[2,41,42]
- Electrically recorded action potentials are accompanied by optically detected movements of the action potential, which mimic the action potential[43,44]
- Nonlinear sound waves/pressure pulses in lipid monolayers can show the main characteristics of nerve impulses[45–48]
- Membrane oscillator theory suggests that ion currents associated with action potential cause vortex phenomena leading to pressure waves in the nerve cell membrane[49]
- Ion channel separation is too great to allow for ion channel interference from adjacent channels caused by ionic charge[50–53]
- Ion channels can be opened by mechanical stimulus[54–57]
- There is deformation of the membrane by activation of ion channels[6,41]
- Entropy (thermodynamic) measurements do not follow the H&H action potential but do follow the APPulse[58–62]
- These findings are supported by detailed mathematical modeling and computational simulations[63–70]

APPulse, action potential pulse; HH, Hodgkin and Huxley.

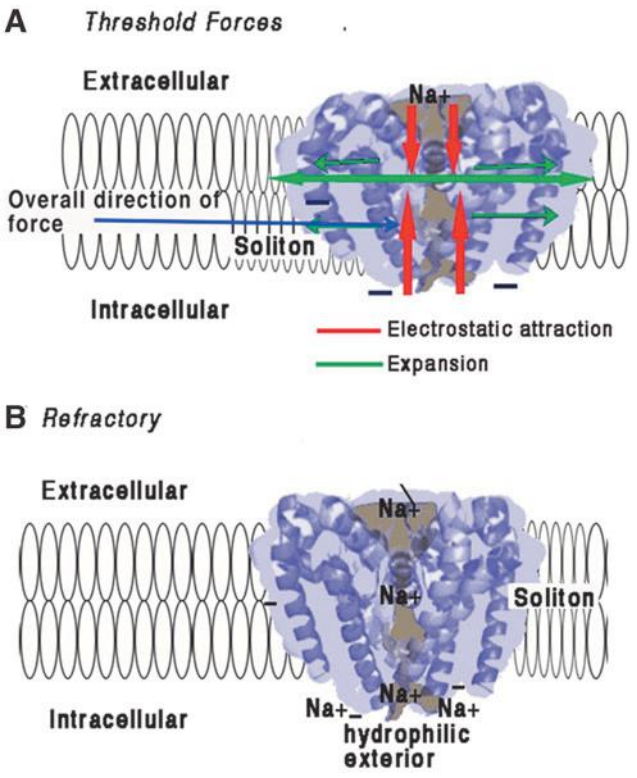

FIG. 2. Instigation of channel opening by the APPulse. **(A)** Pressure from the accompanying pressure wave of the action potential disturbs the ion channel electrostatic seal. Attracted electrostatically charged ions pass through the channel causing it to contract across the membrane. This in turn puts energy back into the pressure wave. **(B)** The ion channel becomes refractory when enough Na+ ions pass through to produce electrostatic equilibrium. APPulse, action potential pulse. Partially reconstructed from Johnson and Winlow[2] and McCusker et al.,[104] used under Creative Commons BY-NC-SA 3.0.

of potassium gates to allow the outward flow of potassium ions. This was then thought to be followed by a decremental wave of depolarization spreading into the inactive region ahead of the action potential to cause further opening of sodium gates, thus allowing action potential propagation along the length of the axon.

Briefly, mechanical surface waves accompany action potential propagation[6,41,42] and these may be necessary to allow opening of ion channels ahead of the action potential since ion channel separation is too great to allow for spread of local current from one ion channel adjacent channels.[50–53] However, in the Hodgkin and Huxley model, action potential propagation depends on flow of charge from one channel to the next across the surface of the membrane, but we have demonstrated elsewhere that the distances involved are too great for this to occur and it would be important for you to see it too[2] because the ''channels are not crowded'' together as shown by Hille in 1992.[105] Furthermore, there is good evidence that electrically recorded action potentials are accompanied by optically detected movements of the neuronal membrane[43,44] and also for deformation of the membrane by activation of ion channels.[6,41] What is more, ion channels can be opened by mechanical stimuli[54–57] as illustrated in Figure 2.

Modulation and Memory Storage

One of the major hurdles to overcome in our understanding of the nervous system is that of memory storage after learning. For example, bacteria such as *Escherichia coli* adapt to changing external conditions,[71,72] have a memory of previous conditions and can modify their direction of locomotion accordingly via their ''nanobrain.''[73] Whether they are capable of associative learning and a form of cognition is open to speculation,[72–74] but of course they do not rely on synapses to store their memory and it is possible that synapses are not the (sole) locus of memory stores in eukaryotes.[86–88]

Within nervous systems, many forms of chemical transmission occur, but of course chemical transmission introduces a time delay, much less so at electrical synapses. It is conjectured from comparative genomic and phylogenetic studies that the earliest transmitters were probably amines, peptides, and gas transmitters such as nitric oxide,[15,75,76] perhaps secreted into water flowing through the organisms. In advanced bilaterians, there is a wide range of transmitters, often used in different ways, but fulfilling similar functions[76] and it has been suggested that that many transmitter pathways may have been transferred from bacteria to animals.[77]

Not all chemical synapses are equal, in that some have greater synaptic weight and thus a greater influence than others on the follower cells to which they are connected, be those excitatory or inhibitory actions. Synapses also provide latency changes through the actions of neurotransmitters. Electrical synapses have reduced latency[78] compared with chemical synapses, but both together produce a spectrum of latencies depending upon their exact construction and location. According to Katz,[79] ''secretion of the transmitter is not synchronous with the arrival of the action potential in the nerve terminal, but lags well behind it'' before even the first quantum of transmitter is released from a synaptic vesicle.[80] However, synaptic delay is modifiable, resulting in synaptic plasticity.[81] This can be related to changes in intracellular calcium concentration, which is important for transmitter release as demonstrated at neuromuscular junctions by Katz and Miledi.[82] Synaptic terminals can be affected by a variety of neuromodulatory agents that may be neurotransmitters, neurohormones, or psychoactive substances[83] and can result in short-term plastic changes such as synaptic facilitation or depression and long-term potentiation or depression of synaptic events. Both the latency and weighting of synaptic responses may be affected[84] and these events can have profound consequences on the behavior of the organism.[83]

Currently, the role of synaptic plasticity in learning and memory is under intense scrutiny by cognitive scientists[85] with the suggestion that memories are molecularly stored within neurons and that plastic synaptic changes in weighting occur only after learning has occurred and been stored in memory. Support for this idea comes from work by on *Lymnaea stagnalis*[86,87] and on *Aplysia*[88] where it has been demonstrated that long-term memory is stored in cell bodies. Thus, plastic changes in synaptic weighting may be ''a means of regulating behavior…only after learning has already occurred.''[85] Such assumptions would not be counter to findings that increased levels of associative learning efficiency are correlated with increased weighting of glutamatergic synapses and downregulated by increased weighting of GABAergic inputs to neurons in the mouse barrel cortex.[89]

From the above it can be seen that the action potential is a plastic phenomenon and that major plastic changes can occur at nerve terminals. The question that needs to be posed is ''how does the nervous system compute the information carried by and between nerve cells and over what sort of timescale?'' Computers tend to work in nano or microseconds while nervous systems have been demonstrated to work on the basis of milliseconds. We will now attempt to resolve this conundrum.

Computation Within Nervous Systems

We have already described the orthodox physiological action potential as elucidated by Hodgkin and Huxley[5] and have suggested that it is accompanied by a soliton pressure wave, the APPulse. We suspect that these events are inextricably connected to one another and together with the computational action potential (CAP) (Fig. 3) they form an ensemble of three inseparable concurrent states. At present it is not possible to determine which, if any, of these states predominates.

Most computational models of the action potential assume that it is a binary event, but it is most likely to be a quantum ternary event,[34] the CAP. It moves along an axon at a defined speed determined by the transmission dynamics of that particular membrane. As with the electrophysiological action potential the CAP has three well defined phases[53]:

- Phase 1: Resting potential of indeterminate length and retaining its integrity under normal resting conditions and actively maintained by membrane pumps
- Phase 2: The spike or digit resulting from ion changes and mechanical activity
- Phase 3: The refractory phase when no new action potentials may occur. Its duration varies accordingly from one axon to another. Unlike the other two phases the refractory phase is an analog variable, which is able to reroute action potentials along different pathways at bifurcations.

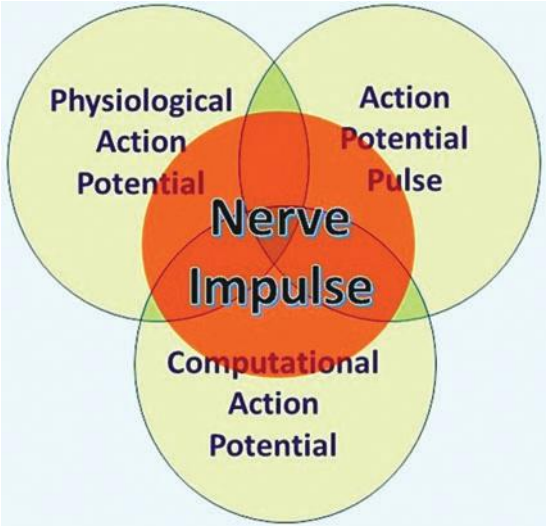

FIG. 3. The nerve impulse may be and ensemble of three inseparable, concurrent states of the action potential. What an observer will perceive depends on their investigational perspective. The physiological action potential is the orthodox action potential described in detail by Hodgkin and Huxley.[5] The APPulse is the mechanical pressure wave for which substantial evidence is presented in Table 1 and the CAP was first described by Johnson and Winlow.[33] CAP, computational action potential.

In computational terms action potential instigation occurs at threshold, not at the action potential peak as we demonstrated in Figure 1. Furthermore, phase-ternary computation occurs when collisions occur between coalescing action potentials across a membrane.[2,33] As a primary source of computation, it is *fast, accurate to microseconds, and efficient.*

The basis of computation is that an outcome reflects inputs for any system. In Figure 4A binary inputs are reflected in the outputs. If the binary inputs change the outputs will change accordingly. The process is not as simple for neurons that compute by differential frequencies. When two asynchronous CAPs collide the temporal component of the refractory period of the leading CAP will annul the second CAP. This is the same as when two opposing action potentials cancel. CAPs are therefore quanta of ternary information computing temporally according to collisions. This temporal computation is important when considering the brain neural network where computation is not timed as in a conventional machine. As shown in Figure 4B it permits temporal computation leading to changes in frequency across the network. In a previous article we explored the shortcomings of artificial network models in neural computation.[90] Most models assume that processing in neural networks work like conventional binary computers, but in our view, this ignores the dynamic structure of real neural networks, operating by phase ternary computation.[90,91]

Quantum mechanics of computation and frequency

The similarities of quantum events and computation between action potentials have recently been discussed[34] and all quantum computation in a network can be described as the result of a combination of collisions of quanta. In an unprogrammed network, like the brain, this process is dependent upon the timing of each event. In a man-made computer, timing between nodes is defined by clock speed and the computation is binary. This results in the computation between nodes being synchronized from one node to the next (Fig. 4A). Nodal transmission is therefore equivalent to one quantum. In a conventional quantum-computer the collisions of quanta are determined by the logic at each node predetermined by programming. In the brain neural network, action potentials pass over the neuron surface as quanta separated by frequency, so more than one quantum can exist within the internodal distance Figure 4B.

The quanta in the brain can be visualized as action potentials, their propagation along a single neuron being binary with a temporal component. Collisions from action potential quanta reveal that the membrane at the point of collision has a refractory period during which no other quanta or action potentials can pass, resulting in their extinction. Recently[33,91] we demonstrated that as each action potential quantum collides at a convergence, the result computes in a phase-ternary manner, due to the membrane's refractory period. At the point of collision on the neuron membrane, each passing action potential exhibits a refractory period thus blocking subsequent action potentials for a specified time.

In the retina the activity of about 130 million light receptors (rods and cones) in each eye are coded down to around 1000,000 neurons in each optic nerve, a huge reduction from input to output that requires error-free computation.[34,91] Consideration of the retinal ''circuit diagram'' provided by Tsukamoto and Omi[92] strongly suggests that the retinal circuitry works by phase-ternary computation, rather than binary analysis.[91] In addition, the timing required for the digital period of computation must take place within 10 μs. Given that the timing of the peak of the action potential is temporally unstable (Fig. 1), it is not possible that this is the source of computation. To compute two action potentials collision must be timed to interfere with 10 microsecond precision or less or computation cannot take place,[91] which implies that it cannot not be explained by a theoretical extrapolation into electrical cable theory.

As we demonstrated above (Fig. 1) threshold is the most appropriate fixed point for neural computation. However, there is no clock in the brain or the retina (which is an extension of the brain) and in the absence of any timing computation cannot take place. In terms of computation by action potentials, there are four key aspects to consider: timing, accuracy, error redaction, and speed of information transfer.

Timing: There is no central clock in the brain

Chronobiology. We are all aware of our daily circadian rhythms but these do not imply that there is central neural clock generating absolute times for our bodies.[93] This becomes obvious to us after a long airflight when these rhythms are disturbed and may take some days to recover, implying that they are entrained by the local environment and that there is no central clock in the brain.[33] Thus, time in the brain or neural network is not the same as the ''absolute time'' available from atomic clocks. However, in computational terms, modern computers are based around the concept of a Turing machine[94] and all contain a central system clock, unavailable in nervous systems, but there is a requirement for computational synchronization within neural networks.

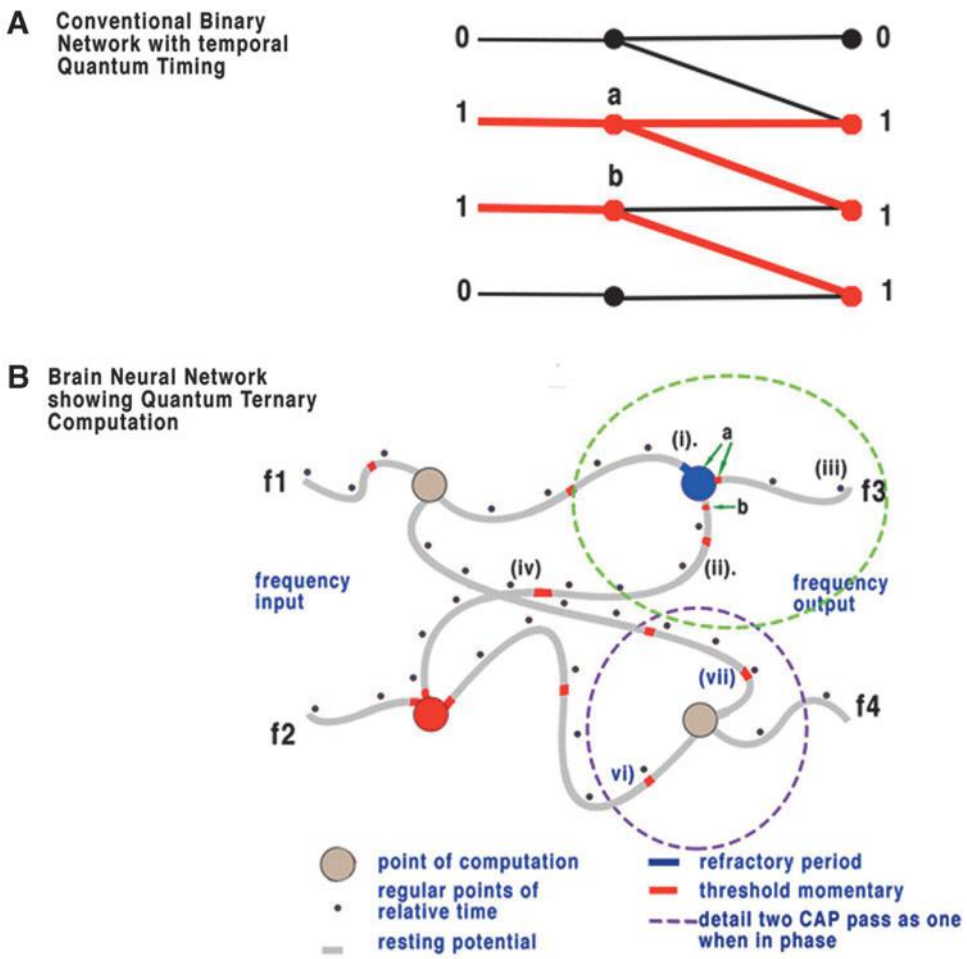

FIG. 4. Computation within networks. **(A)** Illustration of conventional binary computing within a network of eight nodes. Inputs correspond to outputs logically. Nodes are gated by programming and the entire internodal activity is timed to synchronize. In this case (a) there is a single quantum of information shown in *red* extending across the internodal distance. **(B)** Represents a Brain Neural Network with four nodes, these are the convergences of neurons, synapses are part of internodal timing and are not shown. Timing is unrestricted and depends upon the structure of the neuron. CAPs travel along the surface of the membrane. Threshold is represented in *red*. CAP are not restricted to one per internodal length. In the *green circle* a convergence forms a node (i). (a) is a CAP represented by threshold (*red*) and refractory (*blue*). CAP (b) is timed to overlap with a and so will be annulled. The reduction and addition of CAP through the network changes the output frequencies of the output f3. Computation is by differential frequencies and not by base/clock timing. Therefore, changes in frequency of either f1 or f2 in this diagram result in corresponding frequency changes in f3 and f4.

Time and neural plasticity. Given that animal and human neural networks are able to accurately recall events over a lifetime of computation, the formation of memory must occur within a generally stable structure, implying that established structures exist for information processing and memory storage at times when plasticity is inoperative. Thus, computation in these real neural networks must be conducted, not according to contemporary linear time, but in accordance with the timing of plasticity (from milliseconds to years) whether short term, for example, post-tetanic potentiation or long-term plasticity, for example, due to adaptive stress.[95,96] Thus each computation would need to be processed when the network is within a stable state for a very short, but finite moment (in the order of microseconds), before or after which the plastic change is made. Of course, the whole network does not have to be dynamically stable during this short period, only the section relevant to the processing of information that requires processing at that stable time location.

Accuracy

Here we define accuracy in terms of the phase change as action potentials are deflected down different branches of multibranched neurons commonly found in nervous systems, as described elsewhere.[34,97,98] There are many forms of plasticity in neurons, but for accurate computation to take place, as in retinal processing,[91] it is essential that temporal plasticity must be negligible in comparison to temporal timing. Contemporary models of neural networks assume the spike to be responsible for timing, but the model we present[33] indicates that threshold is responsible for timing the next

action potential. Computation thus occurs between the threshold of one CAP and the refractory period of another at positions such as axonal branch points where action potentials can interact with or interfere with one another. Thus, the important attributes of the action potential are threshold, and temporal duration of its refractory period.[2] This implies that computation occurs by interference of one CAP with another often at specific points on the membrane resulting in either annulment of one action potential by another or the generation of trains of action potentials.

Error redaction and noise reduction

Redaction of errors is tied up with accuracy of transmission and is important in both real and artificial brain neural networks. There is substantial noise generated in neural networks,[99] often due to spontaneous activity, but in parallel processing in axons leading to a single neuron such random events are cancelled out by collision with the refractory period of previous CAPs, thus reducing both error and noise in the system.[2,33]

Speed of information transfer and role of synapses

Synapses provide an important role in control of the neural network, but their purpose in computation is to set latencies and to alter the phase relationships of incoming action potentials. Synapses may also act as a slower parallel computational method than phase ternary computation to separate compartments within the neural network. However, phase ternary computation within a neural network is capable of instantaneously, but temporarily, storing information as fast working memory, regardless of any other memory storage or retrieval processes within that network. In the model proposed here, synapses are treated as nodal points in neural networks whose efficacy and phase can be varied. Chemical synapses typically transmit with variable latencies.

We conclude that, in a phase ternary network, memory is a function of associations between all previously recorded events and acts at the speed of the threshold. This is a previously unexplored memory concept. Each synchronization of CAP requires a threshold of no more than 10 μs to digitize the computation. The variation in transmission time for an action potential is many times that. Each trit of information carried by an action potential is carried temporally in the juxtaposition of 10 μs threshold. Where two action potentials propagate on an axon the space occupied carries information in nonary (base 9) containing more information. This increases the memory capability of each neuron. Computation is by single action potentials in parallel in temporal space. It does not require immediate modification of synapses. However, synaptic weighting is modifiable in the aftermath of spike trains[100,101] and is important in learning and memory.[102,103] It is probable that Phase ternary computation within neural networks has general applicability.

In terms of general applicability to both nervous systems and Artificial Intelligence, our belief is that by using a model of the action potential that reflects its multiple modes of action we will be more able to model the mechanisms underlying functional neural networks, be they biological or computational.

Conclusions

- Action potentials are evolutionarily ancient and may have arisen independently on several occasions. In nervous systems they subserve the functions of communication, modulation, and computation
- It is possible that the some or all of these three functions apply to non-neuronal cells such as plant and bacterial cells and in carcinomas, but this hypothesis requires elucidation
- Orthodox Hodgkin-Huxley physiological action potentials appear to coexist with, and may be inseparable from, the APPulse (soliton wave) and CAPs.
- A quantum model of computation explains how action potentials compute within a brain neural network such as the retina and by extension other parts of the brain.

Author Confirmation Statement

W.W. suggested the structure of the article and wrote the preliminary draft. A.S.J. was responsible for the section on computation within nervous systems. We collaborated closely on the final draft.

The co-authors have both reviewed and approved the article before submission.

The article has been submitted solely to Bioelectricity and is not published, in press or submitted elsewhere.

Author Disclosure Statement

No competing financial interests exist.

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6. COVID-19 THE STORY

This is an additional paper not part of the main story.

The role of hemoglobinopathy in COVID-19 pathology. *Fatemi R, Johnson A and Winlow W. Eur. J. Biomed. Pharm. Sci. , 2020, Volume 7, Issue 8, 126-127.*

There are many resources being put into vaccines and drugs to decrease the speed and multiplication of SARS-CoV-2 virions however early on I concentrated on the understanding of the physiologies involved that would lead to the vulnerability profiles of high mortality.

Vulnerability is most acute in subjects over 50. A normal spectrum for illness includes the young. After extensive research I identified a common component in vulnerabilities. The difference is in levels of pool-albumin (not just plasma albumin). This hypothesis also explains vulnerabilities due to obesity, sex etc. We suggest that action be taken to check and raise human serum albumin levels for the over 50's.

*

6.1 Johnson AS, Fatemi R and Winlow W (2020) SARS-CoV-2 Bound Human Serum Albumin and Systemic Septic Shock. Front. Cardiovasc. Med. 7:153. doi: 10.3389/fcvm.2020.00153

I described systemic septic shock as being caused specifically by albumin binding deficiency (ABD) where the products of illness bind to HSA preventing correct binding and colloidal pressure in the interstitial spaces.

Up to November 2022 we have had over 10,500 views and an 89% views rank according to Frontiers. In addition, it is posted on ResearchGate, as are all our other papers.

In reviewing the most recent papers on COVID-19, I realised that much of the evidence I had been seeking was now being published and that all studies were recording declining albumin (HSA) during COVID-19. My work concentrated upon the ability of the body to fight illness. In this respect it is necessary to consider not just the whole body and the separate symptoms but also to go beyond this to the cellular and biochemical mechanisms. I each of these levels I separated the systemic from the local. My search went from whole body to the circulation to individual organs, cellular structures into mechanisms of biochemistry and binding of molecules in solution. This approach has the benefit of being exhaustive when I came to our final conclusions, as I was able in our Sept 22 paper to label and contextualise all vulnerabilities according to levels of HSA binding.

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HYPOTHESIS AND THEORY
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SARS-CoV-2 Bound Human Serum Albumin and Systemic Septic Shock

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The emergence of the COVID-19 virus and the subsequent pandemic have driven a great deal of research activity. The effects of COVID-19 are caused by the severe respiratory syndrome coronavirus 2 (SARS-CoV-2) and it is the underlying actions of SARs-CoV-2 virions on the endothelial glycocalyx that we consider here. One of the key factors in COVID-19 infection is its almost unique age-related profile, with a doubling in mortality every 10 years after the age of 50. The endothelial glycocalyx layer is essential in maintaining normal fluid homeostasis, but is fragile and prone to pathophysiological damage. It is physiologically significant in capillary microcirculation and in fluid distribution to the tissues. Human serum albumin (HSA), the most abundant protein in plasma, is created in the liver which also maintains its concentration, but this reduces by 10–15% after 50 years of age. HSA transports hormones, free fatty acids and maintains oncotic pressure, but SARS-CoV-2 virions bind competitively to HSA diminishing its normal transport function. Furthermore, hypoalbuminemia is frequently observed in patients with such conditions as diabetes, hypertension, and chronic heart failure, i.e., those most vulnerable to SARS-CoV-2 infection. Hypoalbuminemia, coagulopathy, and vascular disease have been linked in COVID-19 and have been shown to predict outcome independent of age and morbidity. Hypoalbuminemia is also known factor in sepsis and Acute respiratory distress syndrome (ARDS) occurs when fluids build-up in the alveoli and it is associated with sepsis, whose mechanism is systemic, being associated with the fluid and logistic mechanisms of the circulation. Glycocalyx damage is associated with changes plasma protein concentration, particularly HSA and blockage of albumin transport can produce the systemic symptoms seen in SARS-CoV-2 infection and sepsis. We therefore conclude that albumin binding to SARS-CoV-2 virions may inhibit the formation of the endothelial glycocalyx by inhibition of albumin transport binding sites. We postulate that albumin therapy to replace bound albumin might alleviate some of the symptoms leading to sepsis and that clinical trials to test this postulation should be initiated as a matter of urgency.

Keywords: human serum albumin, septic shock, coronavirus, endothelial glycocalyx layer, acute respiratory distress syndrome, albumin therapy

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INTRODUCTION

The COVID-19 pandemic has renewed interest in emergent pathogens as a major threat for human health. To date many millions have been infected and hundreds of thousands or greater mortality is expected. Quantitative approaches to the current outbreak are urgently needed to tackle this severe disease.

Coronaviruses are found in many different species of animals (e.g., bats and dromedaries) and can become infectious in humans. Typical spread is by droplets from coughing or sneezing, aerosol, or direct contamination for example through stools. The active virion SARS-CoV survives well and can survive without denaturing for up to 2 weeks in stools after infection. Coronavirus infections are not new, the Severe Acute Respiratory Syndrome (SARS-CoV-2), reported in Asia in February 2003 resulted in almost 9,000 cases with a case-fatality rate of 10%. In 2012, (MERS-CoV) infected more than 2,500 people, killing over 800.

Epidemiological models help policy makers to take decisions. Social distancing has helped mitigate the spread of the epidemic and lockdown of successive geopolitical zones has sequentially reduced both spread and mortality of the disease. Such methods could eradicate the virus but necessitate global co-operation, as does vaccination.

There are many epidemiological unknowns with COVID-19. Infection and mortality rates are still being collated from different sources with varying degrees of accuracy and reliability. Whilst much is uncertain in COVID-19 pandemic, aspects of the disease are well documented such as age related mortality effectively doubling for each 10 years after 50, obesity (1) and the effects on black, Asian and minority ethnic (BAME) individuals (2) which may be unrelated to direct environmental factors and for which there is some evidence of a link to melatonin (3).

One of the difficulties with research into the human body is the transposition of these presentations to cellular physiological and molecular causative events. Of greater mathematical complication is the necessity to understand how within a whole-body cellular infection caused by molecular events lead to systemic injury and death.

In this paper our attention is not in in predicting who dies, but in using this data to find out what corporeal elements have been systemically disturbed and which can account for the differences in mortality across aging, sex and obesity among others. The answer has to be found at the molecular level and as already suggested in the logistical supply of trophic materials to allow the virus to multiply safely without precipitating cell death before the inflection point where the speed of antibody production to remove the virus is enough not to precipitate sepsis. Pathology of systemic sepsis is associated with death in COVID-19 patients (4).

INFECTION

Our understanding of the infection at the cellular level is still poor but we can make assumptions based upon peer-reviewed studies of other infections. Some direct experimentation has already taken place, for example the effects on age from experiments on macaques (5). However, presentation of death related factors and statistical evaluation at the population level of infection and subsequent death rates has resulted in some factors of universal agreement across many countries. A discussion of the exact nature and precision of these evaluations is beyond the scope of this paper, but enough trends from statistical evaluation have been corroborated that allow us to understand both the infection of single individuals and the passage of the COVID-19 virions as they infect single cells and multiply leading to systemic infection and sometimes death.

There have been many suggested criteria examined for prognosis of COVID-19 and these epidemiological markers have been used to selectively profile and isolate members of communities in order to offer protection. The most important of these is the almost unique presentation of age profile, which in epidemiological terms occurs after 50 years of age with a doubling in mortality every 10-years thereafter. Unlike during the 1918 influenza outbreak where prognosis for both the young and the old was one of the major factors of mortality n COVID-19 the majority of deaths are in the over 50s. This difference is crucial in defining the cellular and molecular actions of the virus in infection. Similarly, the high death rates for obese patients and BAME communities may give indications of the mechanism of the virus action as well as the need for protection of minorities. Symptoms of the disease are also indicators for the metabolic distribution of the virions around the body at different stages of the disease. Finally, it must be remembered that at all stages, death is usually presaged by systemic spread of the disease leading to sepsis, which is discussed in detail below. If we are to successfully model the cellular and subcellular of COVID-19, then all forms of systemic stress, such as the multi system inflammatory syndrome (MSIS) Kawasaki type illnesses seen in young children (6) and multi organ failure and sepsis, must be identified.

INFECTION VECTOR

Primary presentation of COVID-19 can include shortness of breath, coughing, loss of smell and taste, and stomach pains, but may include many other symptoms. At the cellular level there are many common events that can be quantified. The infection vector is usually respiratory. SARS-CoV-2 binds to the common angiotensin-converting enzyme 2 (ACE2) (7) cell-surface receptor (8, 9). The virus infects cells using the ACE 2 receptor a precursor in the renin-angiotensin system to angiotensin II a vasoconstrictor. ACE 2 is present in many cell membranes of also all organs and serves numerous purposes within the cell. Infection usually occurs through the lungs, but the ACE 2 receptors are also represented throughout the intestine and nasal epithelia and can also take these routes (5).

ACE2 is prevalent throughout the body. Prevalence of ACE2 makes tissues vulnerable to infection, as the virus requires that receptor be present to enter a cell (7, 9, 10). Virion RNA is then released into the cell where it mimics the cells own messenger-RNA (mRNA). SARS-CoV-2 RNA impersonates the cell's own mRNA instructing the cell to produce the relevant proteins to construct new virions which are then released from

the cell. Antiviral drugs act against this mechanism. The ability of antiviral drugs to slow virion multiplication is an important element against the initial infection. However, deaths ascribed are not due to the SARS-CoV-2 virion but to the consequent disruption to cellular components—this is evidenced by the difference between old and young victims. In older individuals, changing metabolism has rendered them vulnerable. The source of this disruption may only partly be due to the virion itself.

In COVID-19, infection manifests in the alveoli of the upper lungs. Oxygen crosses the alveoli into tiny capillaries that lie beside the air sacs (11, 12). The oxygen is then carried to the rest of the body by hemoglobin in the blood. COVID-19 destroys this process causing oxygen debt, but this may be a secondary symptom. White blood cells release inflammatory molecules in response to abnormality in the cell leading to inflammation and apoptosis (cell programmed death). This is the underlying pathology of pneumonia, with its corresponding symptoms: coughing; fever; and rapid, shallow respiration. Some COVID-19 patients recover, sometimes with no more support than oxygen. Others deteriorate, often quite suddenly, developing a condition called acute respiratory distress syndrome (ARDS) (13). Oxygen levels in their blood plummet and they struggle ever harder to breathe (13, 14). On x-rays and computed tomography scans, their lungs are riddled with white plaques resembling ground glass. Commonly, these patients must be artificially ventilated and their mortality is high. There is no evidence that extracorporeal membrane oxygenation (ECMO) machines that bypass the lungs ameliorate the systemic sepsis and mortality rates of patients from those on a ventilator, probably because the oxygen carrying capacity of the blood is severely attenuated (14). Autopsies have shown that the alveoli were inundated with fluid, eukaryotic material and dead lung tissue (11, 12). Concurrent with this pathology organ failure renal, hepatic and cardiovascular are precipitated by sepsis of the capillary network (15). Subsequent prevalence of clotting, multiple organ failure and all the symptoms of sepsis give a diagnosis of systemic septic shock in almost all deaths.

Most individuals recover quickly from COVID-19 infection, producing antibodies to the disease. However, it should be noted that most patients who do not recover have already either begun to produce antibodies (11) and weak immune system is not a factor in their deaths, but a slow immune system can have fatal consequences. Almost all infected individuals survive the first infection of the virus. Virion levels rise to their maximum and almost all patients produce antibodies. The antibody response is therefore critical to recovery but does not necessarily preclude mortality. Timing is critical between the infection and antibody response and the vulnerabilities of an individual determine their survival.

BACTERIAL VS. VIRAL INFECTION

The basic parameters of bacterial and viral infections differ. Bacteria use their own cellular structures to multiply and do not require the host cellular structures- they can use a primordial pool of material, viruses are restricted to using the host cells own structures to multiply and thus must retain the structural integrity of the host to continue to multiply. Once the cell is depleted of material capable of sustaining the ongoing multiplication deficits will occur disturbing the inherent makeup of cellular components rendering visible to the immune system. For cellular structures to remain intact a constant stream of trophic material must be provided to the cells. Unlike bacteria that can survive and proliferate within a dead environment, viruses like Sars-COVID-19 cannot. The early action of the virus is thus not to damage its own environment and damage to the cell is initially from molecular instability external to normal metabolism and a consequence of cellular logistical inefficiency to provide the requisite materials for the virus to continue its replication. Although often overlooked the virus in early stages of infection is more dependent upon its host than a bacterium. In turn the mechanism of reproduction and subsequent depletion is of is fundamentally important when considering the spread and infection of COVID-19 virions within a body and systemic release and functional damage and subsequent death. If the virion is permitted to replicate where the logistics of cellular structural integrity remains little change will come to the cell. Only when the material for reproduction in terms of molecular quantities of building materials ceases will the mRNA of the virion continue to consume the cellular structure of the host cell. This in turn may change the cellular membrane structure making it visible to antibodies.

APOPTOSIS

The action of cytokine system (16, 17) is not to destroy cell in response to an infection but as a result of cellular incapacity to operate within normal limits as to its function (18). COVID-19 replication depletes the infected cell of material, and usurps its mechanisms. The change from normal activity in turn changes the biochemical nature of reactions at the molecular level starving necessary components necessary to maintain homeostatic integrity. Any action considered normal for the cell does not therefore produce apoptosis, but cytokine release to instigate apoptosis is a result of major cellular damage.

SPEED OF INFECTION AND FALSE IMMUNITY

Viral and host factors play important roles in the course of infection. Critical to life expectancy and prognosis is the speed of infection and subsequent immunity. The development of immunity is critical in the systemic spread of COVID-19 and some assumptions must be questioned as to their scientific acceptability. Immunity can either be from vaccination or from previous exposure. In both cases the body retains antibodies that deteriorate over time (15). Viruses can quickly mutate (19). A COVID-19 vaccination may be ineffective against any new mutation (20). In addition, vaccination against a specific virus will only work effectively while enough antibodies exist in the body with the ability to multiply at a fast enough rate to negate the disease (20). This will decrease with time and immunity will only occur if there are enough antibodies to be replicated

fast enough to overcome the multiplication of virions before they reach the point where apotosis and sepsis takes place. The rules therefore are dependent upon how much each individual is immunosuppressed due to their inherent vulnerabilities and the infection rate. Antibody production does not occur instantly and individuals with immunity are therefore capable of spreading the disease during this process. Thus, all individuals, whether immunized or not, whether showing symptoms or not, become infectious as the immunity commences and this infection continues until antibodies have destroyed every virion present in the body. All individuals therefore are inherent carriers and spreaders of the disease, whether they have immunity or not, by either vaccination or previous infection. As yet, there is no evidence that either vaccination or prior exposure prevents a second exposure from being infectious during the time between infection and destruction of the virus.

In a bacterial infection bacteria divide doubling according to time and medium and occurs exponentially until resources for division are expended. Viruses by contrast do not require division to replicate but use the mRNA and the sarcoplasmic reticulum of the host cell. A single virion may infect a cell and its RNA produce many virion copies unrestricted by the requirement to divide. Virus replication may therefore occur faster than simple exponential division with a single virion multiplying many times up to the point where material for replication is expended. A single virion infecting a cell is therefore sufficient to replicate until the materials for its own division have been expended. Each cell infection also has a critical limit of replication caused by depletion of nutrients which lead to cell death.

ACUTE RESPIRATORY DISTRESS SYNDROME AND SEPSIS

ARDS occurs when fluid builds up in the alveoli and is associated with sepsis (21). Sepsis is life-threatening condition due to a dysregulated host response to infection, which is time-dependent and associated with unacceptably high mortality (13, 22). Mortality remains higher than 25–30% and even higher when shock is present. No effective specific anti-sepsis treatments exist (23).

Sepsis results when an infection triggers a localized inflammatory reaction that causes systemic symptoms of fever or hypothermia, tachycardia, tachypnoea, and either leucocytosis or leukopenia, i.e. the systemic inflammatory response syndrome (24). Severe sepsis is defined by dysfunction of one of the major organ systems. The inflammatory reaction is mediated by the release of cytokines. Cytokines activate the extrinsic coagulation cascade and inhibit fibrinolysis. According to Assiri et al. (25), higher concentrations of the pro-inflammatory cytokine interleukin-6 (26) and the fibrin degeneration product D-dimer (27) were strongly associated with in-hospital mortality in COVID-19. These overlapping processes result in microvascular thrombosis. Thrombosis is one potential factor producing organ dysfunction. Management of patients with sepsis relies mainly on early recognition and correct therapeutic measures. Treatments involve antibiotics resuscitation with intravenous fluids and drugs acting upon targets that are components of the inflammatory response, cytokines such as tumor necrosis factor (TNF), interleukin 1, interleukin 6, or platelet activating factor, or components of the coagulation cascade or vasoactive molecules (28).

The mechanism of sepsis is systemic, being intrinsic to the fluid and logistic mechanisms of circulation, with symptoms originating at the cellular level (29). This pathology manifests in the epithelial cells separating tissues from the external environment and capillary leak syndrome (30). Epithelial cells maintain structural integrity of tissues including the small capillary vessels that surround the alveoli in the lungs and the capillaries of all other organs. Epithelial cells are lined with a gel-like surface of interconnected proteins call the endothelial glycocalyx (31).

ENDOTHELIAL GLYCOCALYX LAYER (EGL)

The endothelial glycocalyx layer has an essential role in maintaining the normal fluid homeostasis of the body (32), but is fragile and can be damaged by a number of pathophysiological conditions and interventions.

The physiology of the endothelial glycocalyx layer has been shown to significantly affect the microcirculation reducing fluidity in capillary flow. Recently the Starling principle of fluid reabsorption and oncotic maintenance in the capillary network has been shown to exist almost exclusively across this layer and not as previously supposed across the capillary system. This has produced the reformed Starling principle (31) that defines fluid distribution more accurately as occurring almost exclusively though this layer. The EGL is therefore essential in maintaining correct oncotic pressure in the capillary bed as well as influencing the adsorption and reabsorption across the capillary membranes. In fluid therapy where osmotic pressure changes, damage may be done to this layer decreasing its thickness and altering its fundamental structural integrity—this is well summarized by Kundra (31). Thus, the EGL maintains the functional integrity and mechanisms supporting not just the physical integrity of the endothelial transport system but also its pathophysiology. In (33) it was concluded that 'Glycocalyx damage may be limited by avoiding hypervolemia and hyperglycaemia and by maintaining a physiological concentration of plasma protein, particularly albumin'. Plasma transfusion has been effective protection of the EGL (34) and has been implicated in preeclampsia (35). Albumin has been proposed for therapeutic targeting of the EGL (36). The main point is that under physiological conditions oncotic pressure is maintained by human serum albumin HSA.

In septic shock damage to the EGL can also cause inflammation of the vascular endothelium and maldistribution of microvascular blood flow as well as the release of the damaging free radical such as nitric oxide (37) which is normally involved in regulation of vascular homeostasis (38), but uncontrolled release is likely to add to tissue and erythrocyte damage, thus reducing the oxygen carrying capacity of blood. The current literature on serology in patients with COVID-19 have reported decreased hemoglobin concentration in these individuals (39). They stated

that coronavirus binds to hemoglobin and frees the iron ions, leading to impairment in the oxygen delivery to the vital organs, release of free radicals and thus elevation in stress, oxidative load, hypoxia and finally heart attack or cardiac arrest may occur (40, 41). There is further evidence for hemoglobin impairments, such as elevated serum ferritin, erythrocyte sedimentation rate, C-reactive protein, lactate dehydrogenase (40, 42). Underlying all of this is damage to the EGL due to modulation of serum albumin by the SARS-CoV-2 virion-albumin binding site as explained below.

HUMAN SERUM ALBUMIN

Serum albumin (43) functions as a significant regulator of plasma oncotic pressure and a transporter of ligands (44–46). It has a half-life in the body of about 20 days and decreases by 10–15% after 50 years of age (47). Importantly 80% of free albumin is contained within the interstitial spaces where it must be assumed to perform the same transport function. Albumin is created in the liver which maintains its concentration, but this reduces after 50 years of age (47). In clinical medicine, serum albumin can be measured via standard serum laboratory testing that measures both free and bound albumin. Free fatty Acids (FFA), melatonin (2, 48–50) and the SARS-CoV-2 virions are transported bound to albumin as are glycolates. Studies on drug interactions confirm both competitive and non-competitive binding (49, 51–54). The role of albumin is well accepted in regulation of hormones (55–57). Research on albumin has provided new insights such as the characterization of the pleiotropic effects of albumin (58). Conformational change to albumin can affect binding of viruses (59). The exact binding sites and therefore the interactions between protein FFA are unknown however the basic mechanism of competitive and non-competitive binding sites have been described. Albumin binds, mostly non-specifically, with each ligand having different affinity for competing sites (60). Glycosylated albumin has long been known to affect platelet aggregation (61) a factor of clotting. Hypoalbuminemia is frequently observed in patients with conditions like diabetes, hypertension and chronic heart failure, and who are statistically most vulnerable to SARS-CoV-2 infection. According to Huang et al. (62) low albumin levels are seen in almost 81% of non -surviving COVID-19 patients and a clinical trial to examine the effects of intravenous infusion of albumin to COVID-19 patients with respiratory insufficiency has recently been registered in India (63). Recently hypoalbuminemia, coagulopathy and vascular disease have been linked in COVID-19 (64) and has been shown to predict outcome independent of age and morbidity (65). Hypoalbuminemia is a known factor in sepsis and ARDS (46, 66–68).

Albumin bonds to ligands by a reversible process where the equilibrium between bound and unbound depends upon the relative concentrations and depends upon ligand. All ligands capable of binding including proteins fatty acids and indeed the SARS-CoV-2 virus are bound in competition for sites to bind upon the albumin molecule as other viruses (45). There are both specific and non-specific areas of binding on an albumin molecule representing different affinities for different ligands (60, 69, 70).

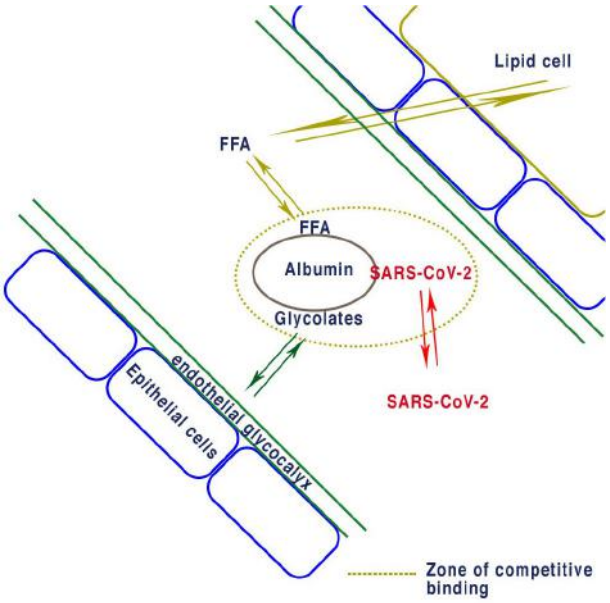

FIGURE 1 | Proposed equilibrium of binding for albumin with FFA determined by lipid cells. Reversible albumin binding of both EGL proteins and FFA determines the concentrations non bound in plasma. If enough SARS-CoV-2 virions bind to albumin they will reduce the concentration of glycocalyx and thus deplete the endothelial glycocalyx layer. Similarly, excess FFA due to obesity causes reduction in the EDL or other ligands bound to albumin.

BINDING OF SARS-CoV-2 TO ALBUMIN

The nature of the SARS-CoV-2 virion-albumin binding site is unknown, but some permanent binding cannot be discounted as proposed in **Figure 1**. At the cellular level the SARS-CoV-2 virus enters the body and uses the cells own apparatus to replicate itself. One single virion can use the mechanisms inherently available to replicate itself in a single cell, and there is no evidence to suggest this process harms the cell. Damage to the cell can only be caused by instability of the cellular mechanisms by depletion of nutrients necessary for replication of the virus and the wellbeing of the cell. Depletion is most likely to occur by expenditure within the cell due to repeated virion manufacture and ejection. The result is an unstable cell vulnerable to apoptosis. The maintenance of the cell is the critical factor and is determined by the delivery of nutrients and the rate they are used. The levels of nutrients supplied by the blood determine this equilibrium.

Spread can be local where one cell releases directly to another adjacent cell or through the extracellular fluid surrounding the tissues leading to systemic release. Initially virions released from a site infection may remain localized. Once they are released, they travel in the blood and lymph bound to albumin.

The immunity conferred on the young must be because of the difference in the systemic environment. Obesity results in a rise in the plasma free fatty acids that are bound to albumin thus

reducing the unbound portion (71). In COVID-19 patients, the level of serum albumin is decreased and the body requires more serum albumin (42). In cases of influenza and other respiratory viruses supplementation with bovine serum albumin was used as a transport medium in a community-wide surveillance of febrile respiratory disease for influenza viruses in order to attenuate the enhanced production of reactive oxygen species such as hydroxyl radicals by neutrophils during an influenza viral infection (72, 73).

CONCLUSION

Unlike influenza where both young and old were affected SARS-CoV-2 mortality increases over 50 years of age. There are very few directly measurable systemic events that change during aging that could be a culprit for death occurring among the over 50's and obese. The reduction of the overall concentration of albumin in the extracellular body fluids is the most likely source of limitation for the transport of substances around the body transporting up to 80% substances. Here we suggest that the albumin complex is inherently the maintainer of homeostatic regulation of essential cellular nutrients as well as oncotic pressure. Our model suggests that albumin transport plays a direct role in the stasis of nutrients that supply the cellular structures that regulate the thickness of the EGL. This provides a direct link from the behavior of cellular SARS-CoV-2 infection, in this case to the destruction of this layer leading to sepsis and general septic shock.

On infection the initial response of infected tissues is to accommodate the virus. This is accompanied by a release of virions into the systemic system where they are bound in equilibrium with Human serum albumin. As infection continues and viral particles expand the ratio between bound and unbound virion-albumin increases until a crisis point is reached. The bound albumin-virion displaces the ligands present on albumin by corresponding amounts. Reduced nutrients provoke cell stress and then apoptosis. Tolerance to the virus thus depends upon, amount of free-albumin. As infection continues the ability of albumin to transport nutrients depends upon the extent of free albumin. Albumin moderates binding the levels of nutrients in the blood. Release of virions into the bloodstream therefore prevents albumin from transporting nutrients into the cells.

We conclude therefore that albumin therapy to replace bound albumin, or strategic addition of HSA to increase the overall concentration in the fluid components of delivery (plasma and interstitial fluid, etc.) might alleviate some of the symptoms leading to sepsis (23, 74). A rise to average albumin levels we predict could alleviate systemic sepsis and prevent death (23, 74). Fluid therapy bundles are available (75–77) containing albumin. However, these are of very low percentages 4% which may become quickly bound and a more long-term sustained approach would be preferable (78).

Fluid therapy is especially important in many surgical situations and this type of physiological intervention whilst not commercially attractive is none the less extremely valid and has a vast history of solid physiological and biochemical research evidence to support it. This is an area of research that is important and apparent in every operation where fluid is given has historically been neglected.

By changing the albumin-bound to albumin-free systemic stress can be reduced with the hope that more lives are saved.

AUTHOR CONTRIBUTIONS

The basic concept was suggested by AJ. AJ and WW worked on the manuscript together with very useful inputs and suggestions from RF. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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6.2 *Johnson AS, & Winlow W. (2021).* ***COVID-19 vulnerabilities are intensified by declining human serum albumin levels.*** *Experimental Physiology. 2021;1–9. DOI: 10.1113/EP089703*

This paper describes how the specific nature of vulnerabilities to COVID-19 are an intrinsic part of COVID19 infection in many patients. I propose that vulnerabilities to COVID-19 may be intensified by a decrease in human serum albumin (HSA) as a ligand carrier for nutrients. A mechanism for COVID-19 vulnerabilities is evident from consideration of ligand carriers such as HSA as intermediaries. We hypothesise that low levels of pool HSA binding, caused for whatever reason, affect the performance of albumin as a carrier protein reducing the availability of nutrients. Hypoalbuminaemia (low HSA) has been implicated as an indicator of COVID-19 and long-COVID-19. The levels of HSA directly affect the immune system and vulnerabilities to age, diabetes and obesity in COVID-19. Any slight reduction in available HSA has profound effects on ligand concentrations in the small capillaries where damage occurs in COVID-19. The clinical implication is that attempts should be made to return HSA to clinical levels to compensate for the additional ligands caused by infection (SARS-CoV-2 virions, antibodies, and cellular breakdown products). Therapeutic albumin is usually given peripherally, and usual preparations are unbound to ligands, but we suggest that a clinical trial of HSA therapy via the hepatic portal vein should be considered. We include a protocol for the umbilical vein.

There has been much discussion over the implications of this work and how the theory on ligand-protein binding functions to maintain nutrients in the periphery. I was investigating how albumin affected the central nervous system but realised that the wider picture of control could have more impactful results. Albumin does not pass this barrier but is envisicled whole by the cell membrane. This process has not been captured in the brain but has been demonstrated in the placenta. In re-examining the foetal growth and evidence from studies it became apparent a similar mechanism exists for COVID-19 as applies to pre-eclampsia and eclampsia. This is described in the following article:

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REVIEW ARTICLE

COVID-19 vulnerabilities are intensified by declining human serum albumin levels

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Abstract

The specific nature of the vulnerabilities to COVID-19 are an intrinsic part of COVID-19 infection in many patients. This paper proposes that vulnerabilities to COVID-19 may be intensified by a decrease in human serum albumin (HSA) as a ligand carrier for nutrients. A mechanism for COVID-19 vulnerabilities is evident from consideration of ligand carriers such as HSA as intermediaries. We hypothesise that low levels of pool HSA binding, caused for whatever reason, affect the performance of albumin as a carrier protein reducing the availability of nutrients. Hypoalbuminaemia (low HSA) has been implicated as an indicator of COVID-19 and long-COVID-19. The levels of HSA directly affect the immune system and vulnerabilities to age, diabetes and obesity in COVID-19. Any slight reduction in available HSA has profound effects on ligand concentrations in the small capillaries where damage occurs in COVID-19. The clinical implication is that attempts should be made to return HSA to clinical levels to compensate for the additional ligands caused by infection (SARS-CoV-2 virions, antibodies and cellular breakdown products). Therapeutic albumin is usually given peripherally, and usual preparations are unbound to ligands, but we suggest that a clinical trial of HSA therapy via the hepatic portal vein should be considered.

KEYWORDS
albumin therapy, COVID-19, human serum albumin, hypoalbuminemia, long-COVID, nutrient distribution, portal system, SARS-CoV-2, virion vulnerabilities

1 | INTRODUCTION

There is now considerable evidence that there are vulnerabilities to COVID-19 exhibited by defined at-risk groups. The vulnerabilities are most severe in the over 50s (Islam et al., 2021), and obese patients are at risk of severe disease (Ho et al., 2020; Huang et al., 2020b). Considerable attention has been concentrated on the infection and destruction of SARS-CoV-19 such that the question of why there are these vulnerabilities has been largely overlooked. The assumption that one cause (the virus) is the purveyor of all the systemic symptoms following COVID-19 infection is inherent in almost all documentation. It is assumed that symptoms and mortality are always a direct result of an increase in virions over time. Recently we described a multipart model based upon peer reviewed material over the last 50 years which results in a far more complex and accurate model of the systems involved (Johnson et al., 2020). The main known factor affecting vulnerabilities to COVID-19 is the availability of nutrient-bound ligand carriers, but present methods of human serum albumin (HSA) therapy are insufficient to prevent morbidity (Boada et al., 2019; Caraceni et al., 2013). Furthermore, a recent paper by Xu et al. (2021, Kheir et al., 2021) demonstrates that low serum albumin levels are a predictor of COVID-19 vulnerability. They studied a cohort of 79 COVID-19

patients combined with a review of electronic laboratory records. This showed that hypoalbuminaemia was common in COVID-19 patients, as has been demonstrated elsewhere (see Johnson et al., 2020 for review), and called for 'dynamic monitoring of serum albumin' to be 'performed during COVID-19 patient treatments': we concur with their viewpoint.

COVID infection usually begins with the lungs but in younger people the infection is often asymptomatic or only localised. Complications that cause what is known as long-COVID with symptoms lasting for many months after the infection, or death, occur at a later stage when other organs become infected until they reach a critical stage. The systemic nature of COVID-19 (SARS-CoV2) when presented as a serious condition indicates that the cause is mediated systemically with infection in multiple organs and subsequent multiple organ failure (De la Rica et al., 2020; Iwasaki et al., 2021).

Immediacy is always present in a clinical situation where a life-threatening illness is involved. SARS-CoV2 has been defined as an acute respiratory syndrome (Larsen et al., 2020) by the early symptoms of the disease with efforts to maintain oxygen concentrations critical to care. Low oxygen levels are only one aspect of respiration and in SARS-CoV2 it is an indication of low lung performance due to damage. The ventilation system of the lungs is intricately tied to the functionality of the rest of the cardiovascular system and the flow of nutrients is a function of blood flow and concentration.

- **What is the topic of this review?**
  Human serum albumin (HSA) a common factor in COVID-19 vulnerabilities.
- **What advances does it highlight?**
  Understanding of HSA capacity, and systemic vulnerabilities to COVID-19. Raising HSA in COVID-19 patients may alleviate systemic injury caused by diminished native HSA binding. A change in fluid therapy administration into the portal system of the liver is proposed to safely raise HSA levels.

2 | HYPOTHESIS

To summarise, HSA is the most abundant human plasma protein. It is the primary transporter of nutrients, fatty acids and hormones in the body and it maintains oncotic pressure. Given that HSA declines with ageing (Figure 1), we hypothesise that this makes older people more vulnerable to COVID-19 infection. If this hypothesis is correct, treatment of COVID-19 symptoms with HSA therapy should be considered, via an appropriate route of administration.

3 | LOW HSA MAY CAUSE THE CELLULAR STRESS SEEN DURING THE SYSTEMIC ACUTE STAGE OF COVID-19

We recently argued that lack of pool HSA that circulates through the endothelial and interstitial structures, bound to ligands, may be the intermediary that leads to cell death and severe illness (Johnson & Winlow, 2020). We demonstrated that the clinical symptoms of SARS-CoV-2 infection occur at frequencies which depend upon available nutrients and infection rates of individual organs within the body. We divided the symptoms into localised and systemic and described the progress of the symptoms of the disease by known molecular events. Unlike the majority of research, which has been concentrated on reducing the virus replication or provision of antibodies (Planas et al., 2021) against variants (Salim et al., 2021), our focus has been on the systemic mechanisms that cause increased morbidity and mortality. This is a novel approach that assumes each infected site within an individual is capable of recovery if correct nutrients are present. The implication is that it is the body's lack of binding resources to contain the virus locally that allows the systemic spread of the virus particularly in vulnerable individuals who are over 50, obese and with underlying clinical conditions.

3.1 | Localised infections

An initial infection occurs locally on an organ system, most usually the lungs. Initial spread may take place systemically with each subsequent organ infected locally. Localised infections are susceptible to both localised and systemic treatments – e.g., by topical sprays (Horby et al., 2021), or by systemic antiviral drugs (Lee et al., 2020a). Each localised infection is controlled according to nutrients and environment. Localised infections lead to localised symptoms.

3.2 | Systemic infection

Nutrients and gases are dispersed throughout the body via all of the systemic fluids. In our model these are primarily the blood (both plasma and red blood cells), the lymph and importantly the interstitial fluids surrounding the cells, all of which form the free body fluids. Our model also includes the cerebrospinal fluid (CSF).

4 | AGE AND VULNERABILITY

What is rarely considered is that in all epidemics including COVID-19 a large proportion of the population survive with little or no long-term effect. The normal physiology of the healthy human body is therefore well-adapted to survive COVID-19 and the norm is for patients to recover. This is easily demonstrated by the survival of young people. Vulnerabilities must therefore be the key to

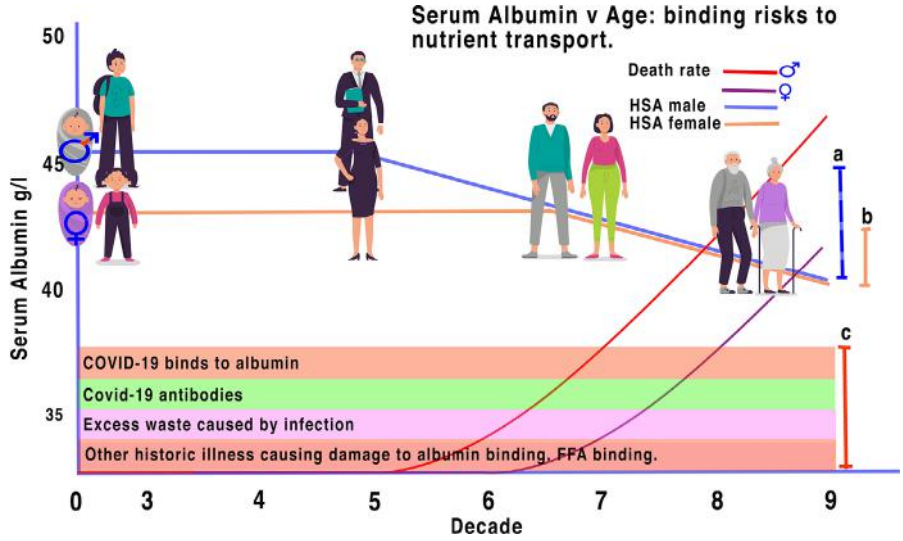

FIGURE 1 Illustrative profile match of albumin decrease with age and risk of COVID-19. Levels of albumin binding changes during ageing making older males more vulnerable after 50 years and females after 65. Serum albumin levels of males (a) decrease with age earlier than those of females (b) (derived from Weaving et al., 2016). SARS-CoV2 virions, antibodies, excess waste and factors from other illnesses reduce the tolerance of unbound albumin further (c). When the number of ligands caused by COVID-19 (c) exceeds that of either the male HSA binding tolerance (a) or the female HSA binding tolerance (b), the ability of HSA to transport nutrients is exhausted. The implication is that as SARS-CoV2 virions enter the system they, and the consequential antibodies and other created ligands, block the natural ability of HSA to bind the correct nutrients causing cellular stress and crisis in the systemic system affecting all organs, leading to excess death rates in both males and females as illustrated (curves derived from data of Islam et al., 2021). Human figures designed by Tartila/Freepik

understanding the mechanisms behind long-COVID, and mortality in COVID-19. Bats are a well-known host to coronaviruses, but they are able to defend themselves (Irving et al., 2021) as is most of the human population. Therefore, individual vulnerabilities are likely to indicate the mechanisms that cause long-COVID-19, and COVID-19 mortality.

A feature of pandemics is their rates of vulnerability and mortality, both related to vulnerabilities in a population, which in turn are formed largely through individual physiologies where each individual has both environmental and genetic variations. Each vulnerability has its own level of harm as demonstrated in population statistics, and those of COVID-19 are now well known (De Larochelambert et al., 2020). Unusually COVID-19 affects the over 50s disproportionately, with children and young adults less susceptible (Crimmins, 2020). The obese are similarly affected (Tamara & Tahapary, 2020). These are common points of reference which we will return to. Thus, COVID-19 does not follow the age profile of previous epidemics like 1918 flu that affected both young and old. A study of the system of nutrient transport led to our previous paper (Johnson et al., 2020) where we discussed HSA transport of nutrients that may affect the endothelial cellular structures – especially of the small capillaries (Figure 2b).

5 | LOCAL PLASMA CIRCULATION

Often the circulatory system is considered a single system, but it is better considered as not one circulatory system based on the heart, but an integrated mechanism of many: the pulmonary cardiac system – regulating gaseous supply; the intestinal–hepatic nutrient system – regulating other nutrients; the renal system – waste removal; etc. Thought of in those terms, each individual organ function has a different locus: lungs – gas exchange, liver – nutrients, kidneys – waste, etc. As plasma circulates, nutrients are removed changing relative pool levels of nutrients. Previously (Johnson et al., 2020) we discussed HSA in terms of its binding to SARS-CoV-2 at the sites of distribution. Recently evidence has shown that hypoalbuminaemia (which we interpret as whole-body reduction in active HSA not just that in the bloodstream) can be used as a direct marker for prediction of vulnerabilities in long-COVID and mortality confirming our hypothesis that hypoalbuminaemia is implicated (Huang et al., 2020a; Kheir et al., 2021; Viana-Llamas et al., 2021; Xu et al., 2021). In these papers the authors suggest using hypoalbuminemia as a clinical marker for serious COVID-19 infection.

6 | THE NUTRIENT CIRCULATORY SYSTEM AND TRANSPORT-PROTEIN MAINTENANCE

Nutrients are provided to the body almost exclusively through the intestines where they pass through the hepatic portal vein into the liver. The hepatic portal vein (Okudaira, 1991) passes quite close to the hepatic artery as it enters the capillary structures ensuring a mixing of nutrient-rich blood and nutrient-poor blood, diluting nutrients, moderating concentrations and binding HSA in the liver to the necessary nutrients to supply the systemic system. It is in the liver that a large proportion of nutrients can be stored in real time to be used for controlled release by various mechanisms, for

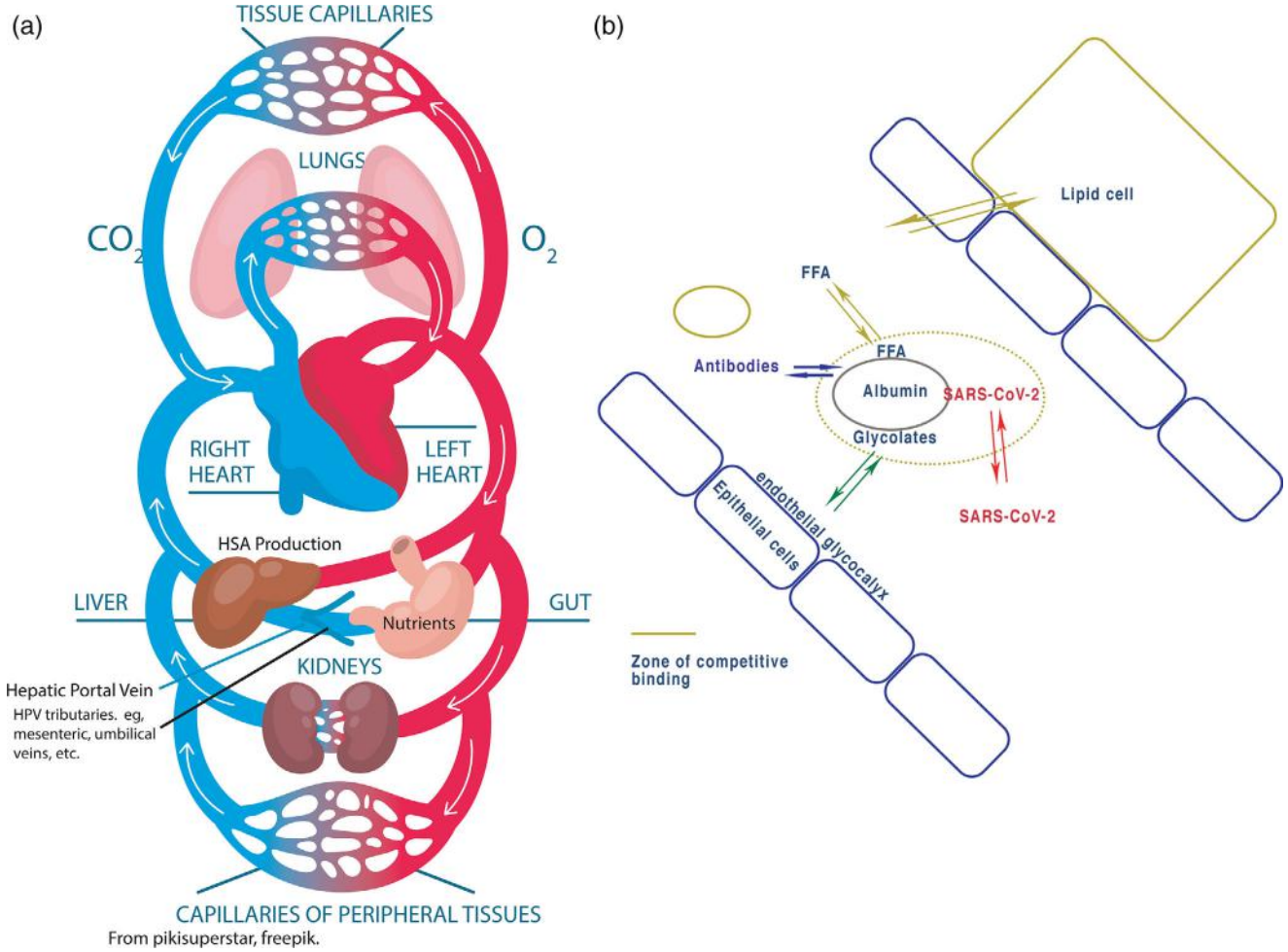

FIGURE 2 Illustration of direction of flow of nutrients from the gut and ligand exchange in the capillaries. (a) Circulatory system showing direction and flow of nutrients from the intestine through the heart and lungs. Fluid therapy to the periphery of unbound HSA will flow to the heart and then is concentrated in the lungs and may make many circulations before re-entering the liver . HSA entering the liver is charged with ligands before circulating. Thus fresh, unbound HSA could be introduced into the hepatic portal vein using standard techniques as described in the discussion. (b) At the capillary level, concentrations, and therefore delivery of ligands (FFA – free fatty acids), are determined by relative concentrations across cells. Competition exists to maintain equilibrium and any outside element such as SARS-CoV2 will interfere with this balance. (From Johnson et al., 2020 – reproduced under the Creative Commons Licence.) (a) modified from pikissuperstar freepik

example insulin modulation of glucose and glycogen. The liver also produces and regulates the concentrations of many other nutrients in the plasma including HSA (Lee et al., 2020b). Liver function tests have recently revealed that 33% of COVID-19 patients suffer from hypo-albuminaemia (Weber et al., 2021) and patients with acute liver injury are known to suffer from acute hypoalbuminaemia (Signorini et al., 2021). Thus, acute damage to the liver may have lethal consequences following COVID-19 infection. From the liver, plasma rich nutrients, modulated by the liver (Levitt, & Levitt, 2016), maintain relatively stable concentrations of ligands, passing into the hepatic vein, vena cava and heart where nutrients are further diluted. Importantly for our research, nutrients then pass into the capillaries of the lungs before continuing back to the heart and then on to the tissue capillaries.

In the tissue capillaries, nutrient concentrations change in the plasma as it passes from arterial to venous as nutrients are absorbed. Boden (2008) showed that levels of fatty acids in the plasma corresponded to levels of fat in cells. The levels of nutrients in the plasma and in the capillaries decrease according to relative usage in adjacent cells. The HSA levels are set between the plasma and intra-cellular levels. The level of passively transferred nutrients in tissue corresponds to the relative level in the plasma in adjacent cells (Levitt & Levitt, 2016). Nutrients that do not flow directly into cells pass into the interstitial spaces between cells. The flow of plasma in interstitial spaces is greatly reduced, for example causing the half-life of large molecular structures like HSA to be 20 days (Moman et al., 2020). Large molecules like HSA, the 'sediment' (Moman et al., 2020), travel more slowly than the plasma, hence a half-life of 20 hours. HSA circulation is thus separate from the fluid plasma. Interstitial fluid eventually passes into the lymph, finally re-joining the venous flow to the heart via the lymphatic ducts. The implication of the lungs being fed first is that any large nutrient like HSA will remain in the lungs, where up to 80% may remain in the interstitial fluid (Weaving et al., 2016).

7 | PLASMA TRANSPORT AND MODULATION OF LIGANDS

A large proportion of ligands carried in the blood are not in the fluid plasma but become bound reversibly to each other and to large ligands. HSA, prealbumin and gamma globulins are some of the ligand carriers (Collins, 2001; Levitt, & Levitt, 2016; Moman et al., 2020). However, HSA has by far the highest concentration and must be the first candidate for examination.

The reversible nature of this binding is such that a large ligand carrier molecule can carry many ligands. Ligands are carried therefore in two separate mechanisms in the plasma: in the liquid portion and bound to large ligand carriers such as HSA. Because many ligands like glycolates are almost entirely bound to carrier proteins such as HSA, ligands carried in the fluid are many times less concentrated than are the protein-bound ligands. This has the following advantages.

1. Per volume, plasma can carry a far greater concentration of ligands than carried in just the fluid.
2. The relative oncotic pressure change produced from the ligand binding maintains pressure.
3. There is a modulatory effect when ligand concentration diminishes in the fluid portion in the capillaries; replacement of ligands automatically follows from the ligand carriers, thus maintaining a relatively stable concentration. For example if 10 times as many ligands exist bound to a carrier as in the fluid, if all of the fluid ligand is removed and the equilibrium restored, the concentration will only have dropped by 1/10 of its value. Conversely a drop of 1/10 of the carrier protein will result in a drop in total of 1/10 of total ligand concentration. This provides a buffer where the total content of the ligand in plasma is related to the concentration of ligand carriers. High ligand carrier concentrations have little effect, but any loss results in an immediate fall in ligand availability: this is most relevant to individuals whose HSA levels are close to the physiological minimum.

Any change in the status of the ligand carrier will result in changes in the plasma concentration of the ligand, affecting its mobility and in consequence its actions at its destination. Many of these ligands have homeostatic regulation of their own and form their own circulatory systems, HSA, for example, binds with glucose. This is not a simple system as multiple ligand carriers will have different affinities and binding capacities for their respective ligands. For example, although prealbumin only represents a tiny fraction of binding compared to HSA, it may bind preferentially to some ligands, which may be critical. In the case of COVID-19 we present evidence of how this transport system functions in relation to HSA.

8 | HUMAN SERUM ALBUMIN

Age and sex variation of HSA concentration confirms that 'The mean population serum HSA concentration increases to peak at around age 20 years and then decreases with increasing age. Initially higher, values in females decrease more rapidly but become close to male values at 60 years' (Weaving et al., 2016). HSA constitutes almost 50% of all protein found in the blood. It is synthesised in the liver and has a half-life of 20 days in the plasma (Moman et al., 2020). Up to 80% of HSA is not contained in the plasma but in a serum pool of free body fluids: the lymph, interstitial fluids, CSF (Suárez-Gonzalez et al., 2020), there is a pool for the placenta, the testis and ovaries, everywhere there is a barrier, where it feely circulates and mixes with the plasma with a half-life of about 8 h (Levitt & Levitt, 2016). Hypoalbuminaemia can also lead to hypertension via the immune system, which is dysregulated in hypertension and SARS-CoV2 infection (Drummond et al., 2019).

COVID-19 complications occur at the systemic stage of the infection and then lead to the integral collapse of the endothelial cellular structure leading to the more serious systemic symptoms and death. We propose that this is caused by a similar system to that of sepsis (Johnson et al., 2020; Roger, 2021; Stasi et al., 2021), but which we have redefined more specifically for operational purposes as being 'the symptoms caused by an inoperative systemic nutrient transport system'. We provide evidence that the main cause of this is insufficient unbound HSA. Although other proteins may be involved, 80% of all ligands are carried in the blood on HSA (Moman et al., 2020). We have identified a reduction in active HSA as being the common point in sepsis and in SARS-CoV-2 major systemic failure. In brief:

1. HSA is reduced by up to 33% in patients over 50 (De la Rica et al., 2020; Ghahramani et al., 2020; Weaving et al., 2016; Weber et al., 2021).
2. Low Serum HSA predicts severe COVID-19 (Huang et al., 2020a; Kheir et al., 2021; Viana-Llamas et al., 2021; Xu et al., 2021) and can be used as triage for serious illness (Viana-Llamas et al., 2021; Xu et al., 2021).
3. Hypoalbuminaemia is also a known factor in sepsis, acute respiratory distress syndrome (ARDS) and COVID (Roger, 2021; Stasi et al., 2021).
4. Eighty per cent of HSA resides in the pool of interstitial spaces and lymph. It is the main provider of proteins forming the endothelial glycocalyx layer – the inherent stabiliser of endothelial cell connectivity maintaining the structure and placement of endothelial cells (Johnson et al., 2020).
5. Any externally applied ligand may bind competitively to HSA and displace the transport-nutrients necessary for the cell. These include viruses like SARS-CoV-2 and antibodies, both of which reduce binding capacity for other ligands as COVID-19 reaches a critical point (Johnson et al., 2020). Levels of available binding decrease upon increase in levels of free fatty acids (Boden, 2008) – and the SARS-CoV-2 virions. Reduction in binding leads to sepsis.
6. Plasma HSA may also affect gaseous nutrients; for example, haem is also transported on HSA along with units of oxygen (Ascenzi et al., 2015).
7. Low serum HSA level predicts mortality in dialysis patients (Mehrotra et al., 2011).

Without knowledge of how drugs operate as ligands when transported, topically localised drugs may increase recovery time. However, in HSA-compromised individuals, systemically applied drugs will inevitably have greater effects on symptoms and mortality (Guzik et al., 2020).

Levels of HSA in the free body fluids, including interstitial fluid and the CSF, affect the concentrations of proteins within a localised area. We suggest that the presence of SARS-COV-2 binding to HSA displaces and reduces the ligands available for transport. This depletes nutrients at their sites of requirement. This indicates that, since HSA is the main transport mediator of the body, its relative concentration is a part of the regulatory mechanism that defines cellular integrity (Apte, 2020). Transport of ligands by other sources such as gamma globulins also acts in competition with HSA. However, HSA administered in high doses has been examined for its therapeutic value in models of some CNS diseases. In models of ischaemia (e.g., global, transient focal, or permanent focal) or traumatic brain injury, administration of HSA resulted in protection (LeVine, 2016).

8.1 | Other properties of HSA add evidence to the hypothesis

It has been demonstrated that when more ligands are present after injury, HSA concentration is increased (Ishida et al., 2014; Pérez-Guisado et al., 2013). Indications in COVID-19 implicate degraded liver function maintaining or reducing HSA levels (Crea et al., 2020; Guzik et al., 2020; Paliogiannis et al., 2020). There is evidence that declining HSA is associated with nutritional risk, physiology and system inflammation (Almasaudi et al., 2020; Iba et al., 2020; Iwasaki et al., 2021) as well as rheumatic heart disease (Wei et al., 2017). Long-term administration of human HSA improves survival in patients with cirrhosis and refractory ascites (Di Pascoli et al., 2019) and hospital mortality (Akirov et al., 2017).

It is important to note that HSA maintains the fluid balance in the body and any condition that results in a decrease in plasma volume will cause falsely elevated HSA levels (Ishida et al., 2014). The serum HSA test only looks at the levels of HSA in a person's blood, not in the rest of the body fluids. HSA concentrations rise slowly during nutritional therapy (refeeding) and in patients recovering from stress (Caraceni et al., 2013). Changes in HSA can be acquired by at least 2–3 weeks of nutritional intervention. Diseases such as COVID-19 cause the liver cells to lose the ability to synthesize HSA (Guzik et al., 2020; Paliogiannis et al., 2020).

In our hypothesis (Johnson et al., 2020), we proposed that the serious symptoms of COVID-19 are caused by hypoalbuminaemia (Gounden et al., 2020; Larsen et al., 2020). Maintenance of systemic homeostasis of nutrients requires a certain level of HSA, which may become saturated on addition of SARS-CoV-19 virions as well as subsequent antibodies that bind to its structure. Thus, the concentration in the blood plasma is only one representation of effective HSA, because any other previous infection or illness that affects blood concentrations of ligands bound to HSA will have moderating effects on cell physiology, e.g., insulin, fatty acids, which increase in diabetes (Boden, 2008), many antibiotics and other drugs. Furthermore, recent infections will generate antibodies that will bind to HSA.

9 | DISCUSSION

Here, we have explained how HSA production and subsequent binding to ligands is managed by the liver and explain the potential positive effects of HSA therapy applied to the portal system. The evidence we have presented suggests that COVID-19 presents initially as a symptomatic respiratory disease, which then distributes systemically causing organ failure. We have also demonstrated that organ failure in COVID-19 is due to localised hypoalbuminaemia caused by competition on HSA-binding sites by SARS-CoV-19 and the body's immune response. We have also shown how HSA binding capacity can be influenced by the body's own decrease in the over 50s: obesity by the action of FFA competition for albumin. Other comorbidities, such as organ damage, will change the equilibrium of bound ligands to carrier proteins affecting the same systemic transport system. The nutrient transport system of the human body is constructed so that only a very minor quantity of nutrient will be transported in the fluid portion of the plasma; almost all ligands are transported bound to ligand carriers creating large buffers of competitive nutrient ligands in equilibrium between the fluid and the 'sediment' containing ligand carriers. HSA is by far the largest ligand carrier.

The circulation of HSA begins in the liver where nutrient supply into the plasma is moderated. At this point HSA is pre-bound, and in healthy subjects all ligands are in an equilibrium, which will transport ligands to their sites of absorption, in the correct concentrations for heathy cellular activity. HSA that leaves the liver is 'loaded' to its optimal extent with a variety of ligands representative of the state of the liver and its supply (Figure 2a). As HSA passes through the circulation and especially the small capillary and interstitial spaces, the relative concentrations of ligands bound to HSA change as the cellular structures acquire ligands. This will change both current binding and oncotic pressure according to what nutrients are released and waste binds to HSA and other carriers. Any slight decrease in HSA concentration will produce a corresponding large decrease in available ligands especially in the distal capillaries and if HSA is near minimum. This will cause many of the blood markers (Kim et al., 1999) for inflammation and autoimmunity (Lee et al., 2020b) to change value as has been seen in COVID-19 (Thwaites et al., 2021). These changing markers may be a signal of HSA binding deficiency as SARS-CoV2 virions displace other ligands from binding sites. Any foreign ligand or destabilisation of the HSA binding will reduce the active level of HSA further as it is charged in the liver or interacts throughout its circulation. When a SARS-CoV2 virion binds to HSA, this reduces the binding activity for other ligands, which then have a corresponding drop in concentrations in the distal small capillaries.

10 | HSA THERAPY AND THE APPROPRIATE SITE OF HSA ADMINISTRATION

Infection in COVID-19 is usually via the lungs, which contain a large proportion of HSA; low HSA in the system will therefore preferentially bind SARS-CoV2 virions. HSA remains in the body with a half-life of 20 days, much of it resident in the interstitial spaces from which it moves slowly. The symptoms of long-COVID follow a similar pattern expected from hypoalbuminaemia caused by SARS-CoV2 binding to HSA, which remains in the interstitial spaces. One method of reducing the risk of vulnerabilities to COVID-19 might be HSA therapy (Mani Mishra et al., 2020; Mendez et al., 2005), but we need to know the most appropriate site of administration as this is critical.

Currently HSA therapy is given into a peripheral vein that then flows to the vena cava, heart and then lungs. HSA given in this manner is effectively in an unnatural state as any binding of HSA is unrepresentative of the ligand–HSA equilibrium formed on loading in the liver. Any addition of HSA into a peripheral vein is preferentially absorbed into the interstitial spaces of the lungs for the half-life of HSA in the pool, about 20 h, before passing to the rest of the body, concentrating the unbound albumin in one place. Of course, binding will also occur between the administered HSA and ligands in competition with other HSA already bound, but this will further distort the nutrients delivered with potentially many days before re-equilibrium throughout the systemic system; this problem is exacerbated with higher HSA concentrations and a longer time period. On administration, the HSA passes into the capillaries and is slowed in movement as it passes into the interstitial spaces causing any new HSA to congregate in the lungs. The equilibrium of the ligands bound to existing HSA in the body changes to reflect the state of the new unbound HSA, reducing the overall concentration of ligands in the plasma as described above. For ARDS diseases like COVID-19 this is especially problematic when the lungs are already inflamed and nutrients low. There is also a risk that unbound HSA will preferentially bind to SARS-CoV2 virions in the lungs and become systemic. A more appropriate site of administration is therefore required.

Safely raising the levels of healthy bound HSA in plasma will depend upon the site of administration and the form in which it is given: whether it enters the liver to be charged by nutrient-ligands or the lungs where it may cause damage. HSA to be given correctly would require pre-ligand-binding at least representative of the ligand equilibrium formed in the liver where binding takes place. The liver is a highly evolved organ and its capacity for HSA synthesis and moderation of correct nutrient ligands must not be underestimated; in a functioning liver any amount of HSA should be preferentially bound to the correct ligands. Ideally, then, HSA should be given into the portal system via the hepatic portal vein or its tributaries (Okudaira, 1991) or the umbilical vein, which is accessible in most adults and 'can be cannulated in a superficial position in the upper abdomen' (Braastad et al., 1967). Other entry sites to the hepatic portal vein exist via catheterisation through jugular, femoral or humeral veins (Butzow & Novak, 1977; Lebrec, 1991). Giving HSA to the portal system should therefore provide greater effectiveness and control over administration of HSA for both oncotic pressure and ligand binding, thus providing a more stable environment in which to raise HSA. The evidence presented demonstrates that clinical trials should be carried out to test this hypothesis as soon as possible with the aim of reducing vulnerabilities to COVID-19.

11 | CONCLUSION

There is evidence to support the hypothesis that low binding of nutrients in the HSA pool may be a contributor to severe COVID-19 at the systemic stage. Raising the HSA binding in the pool level causes complications by disturbing both the oncotic balance and also the equilibrium of ligands bound to HSA carrier proteins. Thus, HSA should be given bound to ligands or through the hepatic portal vein and tributaries such as the umbilical vein at more than minimal levels. Addition of HSA directly to the liver has the advantage that the resultant albumin delivered to the systemic system will have been bound to appropriate ligands. This process may help alleviate severe COVID-19 symptoms. We call for detailed clinical studies of this hypothesis.

COMPETING INTERESTS

No competing interests.

AUTHOR CONTRIBUTIONS

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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6.3 Johnson, A, Winlow, W. Pre-Eclampsia, Hypoalbuminaemia and Albumin Therapy. *Eur. J. Biomed. Pharm. Sci. 2021, 8, 75–78.*

Albumin is a transport protein for other ligands: its presence in the plasma modulates nutrient supply to tissues. During pregnancy the foetus metabolises albumin passed across the placenta causing a depletion that cannot always be maintained by the maternal pool of albumin. In cases of poor nutrition, a cellular nutrient crisis may occur during fast foetal growth leading to maternal albumin deficiency and consequent nutrient loss throughout the systemic system. We suggest that to correct this loss and provide properly ligand-charged nutrients albumin should be infused into the portal vein system of the liver and not the periphery ensuring that new albumin is passed through the liver before entering the systemic system.

This paper describes how a low albumin crisis can occur during pregnancy due to the foetus. Albumin only travels in one direction in the placenta, being completely metabolised by the foetus. This produces a deficit during maternal fasting or illness that would affect albumin binding. This mechanism also provides evidence for the hypothesis that COVID-19 vulnerabilities are caused by low albumin.

Extensive research was carried out across all the subjects included in this paper. It is necessary to understand the flow of HSA and the vulnerabilities to each organ at the level of cellular function to understand how the can effect ABD. This includes almost all the organs of the human body. In addition, both evolutionary and developmental aspects of the body and growth were considered. There is a section both explaining how HSA in the central nervous system is maintained and how that maintains nutrients.

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PRE-ECLAMPSIA, HYPOALBUMINAEMIA AND ALBUMIN THERAPY.

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ABSTRACT

Albumin is a transport protein for other ligands: its presence in the plasma modulates nutrient supply to tissues. During pregnancy the foetus metabolises albumin passed across the placenta causing a depletion that cannot always be maintained by the maternal pool of albumin. In cases of poor nutrition, a cellular nutrient crisis may occur during fast foetal growth leading to maternal albumin deficiency and consequent nutrient loss throughout the systemic system. We suggest that to correct this loss and provide properly ligand-charged nutrients albumin should be infused into the portal vein system of the liver and not the periphery ensuring that new albumin is passed through the liver before entering the systemic system.

Pre-eclampsia affects from 3% to 14% of pregnancies worldwide and is a major cause of maternal and perinatal morbidity and mortality. There is already considerable evidence that pre-eclampsia may be intensified by a decrease in human serum albumin (HSA) (Gojnic et al 2004, Martell-Claros et al 2019). We have shown in recent review articles (Johnson and Winlow 2020,Johnson and Winlow 2021) that levels HSA and other ligand binding proteins in blood plasma are intrinsically linked to the levels of nutrients provided to the cells of epithelia where most damage occurs (Johnson and Winlow 2020) during the systemic stages of COVID-19 infection.

In applying our hypothesis on transport proteins from our work on albumin and COVID-19, a mechanism for the causation of the symptoms of pre-eclampsia became apparent. We provide evidence that pre-eclampsia is probably due to a crisis of hypoalbuminaemia caused by foetal metabolism – HSA produced by the mother's liver is metabolized by the foetus, but none is returned to the maternal circulation. Although HSA and its binding properties have been known for many years a full consideration of how binding proteins, such as HSA, transport vital nutrients systemically is not often considered, except as markers for illnesses like cancer. There is now considerable evidence to suggest that HSA levels are critical in maintaining the nutrient balance and concentrations in the target cells (Rabbani et al 2021, Johnson and Winlow 2020).

In the pregnant female a crisis of severe hypoalbuminaemia can occur due to the mechanisms of HSA maintenance between the mother and foetus. To supply the foetus with nutrients the HSA-complex does not pass the placental barrier (PB) but is envisicled by attachment to the protein clathrin (Lambot et al 2006). HSA is not returned by the foetus and not returned to the pool of the mother. The mother's liver therefore must provide the bulk of foetal HSA for the plasma and free-body-fluid pool where most HSA resides.

The foetus metabolizes HSA completely not returning it to the placenta or the mother's vena cava, foetal pool HSA is then circulated through the foetal liver and placenta. This may produce hypoalbuminaemia especially during rapid growth of the foetus, or poor nutrition of the mother or illness. Diet and nutrition are known risk factors for preeclampsia (Cao et al 2020). We submit that it is probably hypoalbuminaemia caused by foetal oversupply at a time of nutrition deficit that causes the symptoms and presentation of pre-eclampsia.

We hypothesise that low levels of pool HSA binding, caused for whatever reason, affect the performance of HSA as a carrier protein (CP) reducing the availability of nutrients. HSA is created in the liver of the mother where it is bound to the suitable nutrients to supply the plasma. It is unclear whether the foetal liver supplies any of its albumin requirements – the regulation is therefore determined by the mothers' liver. The clinical implication is that attempts made to return HSA to clinical levels must be directed at the portal system of the liver or must have HSA already bound to the appropriate proteins. Our hypothesis involves considering albumin and other binding-proteins as being part of the liver

circulatory system. In this model the regulation of albumin is defined mainly by the oncotic pressure within the portal vein of the liver. Albumin formation in the liver is formed in equilibrium with its precursors by the hepatocytes, the levels of albumin and corresponding precursors varying according to concentrations of precursor components that are catalysed by the correct portal vein pressure. It has long been known that portal vein oncotic pressure is related to HSA concentrations which in turn defines fluid volume. This process is reversible with the liver able to use and re-metabolize components for storage and other protein synthesis. In turn the availability of albumin permits the free control of essential components to the cellular structures (Johnson and Winlow 2020, Johnson and Winlow 2021). This produces an immediate hypoalbuminaemia, especially when the patient is low on pool HSA with subsequent collapse of the systemic system. Therapeutic albumin is usually given peripherally, and most preparations are unbound to ligands. Therefore, we suggest that a clinical trial of HSA therapy via the hepatic portal vein should be considered and studies should be performed to evaluate better preparations of HSA. HSA and other carrier proteins are intrinsically implicated in the systemic spread of COVID-19 (Johnson and Winlow 2020, Johnson and Winlow 2021) but protecting the body against the vulnerabilities of pre-eclampsia by using the portal structure is a new untried technique, which, if it works, should reduce harm directly.

We conclude that the reason infusion of HSA has historically led to unpredictable results is that using a peripheral point of entry leads directly to unbound-HSA lingering in the HSA pool. Addition of HSA must be given to the portal system so that nutrients and colloidal pressure may be correctly maintained within the systemic transport system. This area of research has been neglected and we feel very strongly it should be included in further research on pre-eclampsia. It is our hypothesis that properly increasing available pool HSA will eliminate many symptoms of pre-eclampsia. This can be achieved by pre-binding the carrier protein HSA before addition or adding to the portal vein instead of a peripheral vein to enable the natural binding of liver ligands to HSA to occur and for correct modulation of colloidal pressure. Infusion of HSA from a peripheral vein should take place through the portal system to ensure correct management both of nutrients but colloidal pressure.

Author contributions

ASJ conceived this work based on our previous reviews on Covid-19. We designed, refined and approved the submitted version of the manuscript together.

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Conflicts of interest

The authors declare no conflicts of interest.

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6.4 Johnson AS, Polese G, Johnson M and Winlow W. **Appropriate Human Serum Albumin Fluid Therapy and the Alleviation ofCOVID-19 Vulnerabilities: An Explanation of the HSA Lymphatic Nutrient Pump**. COVID 2022, 2, 1379–1395. https://doi.org/10.3390/covid2100099

COVID-19 and long COVID-19 vulnerabilities may be caused indirectly by albumin binding deficiency (ABD), which can be corrected by the correct administration of human serum albumin (HSA). The liver is the primary site of nutrient regulation and fluid volume maintenance; control of both is by changes to albumin concentration. In healthy subjects, the HSA lymphatic nutrient pump (HSALNP) ensures continual pumping of nutrients from the liver and that nutrients are appropriately distributed to organs. Nutrients are delivered to cells according to the availability of binding to HSA. The HSALNP, therefore, maintains the correct nutrient and colloidal pressure balance in all tissues independently. In unhealthy tissues, following COVID-19 infection, the passage of HSA/nutrients through the interstitial spaces and lymph will be impeded. Fluid therapy into the periphery leads to the dilution of essential nutrients attached to the protein carriers such as albumin. The levels of albumin being charged by the liver with nutrients is critical in maintaining immune stability by maintaining nutrient support and colloidal pressure of the cellular structures. The site of HSA binding by the liver is of great importance, and direct infusion of albumin into the hepatic portal vein is the most appropriate method of maintaining colloid pressure and cellular nutrient levels.

To attract attention to my discoveries we wrote a letter to the editor which became a mini review for *EC Pharmacology and Toxicology*. At this stage my theory developed from whole body physiology as it is now clear that levels of albumin act primarily systemically across all free body fluids maintaining firstly colloidal pressure which then triggers the adaptation of blood cells, nutrients, and other ligands. This control is exclusively by the liver and the evidence I have provided shows addition of albumin to the may bey used to regulate all these processes. This is an added tool for clinicians in almost all disciplines of medicine and sport as it may define the ability for acclimatisation to altitude and exercise as well as the general health of the body.

There are many questions left to answer and that must be left for experimental physiologists to determine, one of which is the response to insulin transport in recovery. In infusion of albumin there is a physiological limit to the amount defined by the health of the liver, other ligands may have to be added to facilitate correct binding.

If the experiment is performed to raise albumin whatever the result my work will still have created a line of research that will likely continue.

COVID

MDPI

Review

Appropriate Human Serum Albumin Fluid Therapy and the Alleviation of COVID-19 Vulnerabilities: An Explanation of the HSA Lymphatic Nutrient Pump

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Abstract: COVID-19 and long COVID-19 vulnerabilities may be caused indirectly by albumin binding deficiency (ABD), which can be corrected by the correct administration of human serum albumin (HSA). The liver is the primary site of nutrient regulation and fluid volume maintenance; control of both is by changes to albumin concentration. In healthy subjects, the HSA lymphatic nutrient pump (HSALNP) ensures continual pumping of nutrients from the liver and that nutrients are appropriately distributed to organs. Nutrients are delivered to cells according to the availability of binding to HSA. The HSALNP, therefore, maintains the correct nutrient and colloidal pressure balance in all tissues independently. In unhealthy tissues, following COVID-19 infection, the passage of HSA/nutrients through the interstitial spaces and lymph will be impeded. Fluid therapy into the periphery leads to the dilution of essential nutrients attached to the protein carriers such as albumin. The levels of albumin being charged by the liver with nutrients is critical in maintaining immune stability by maintaining nutrient support and colloidal pressure of the cellular structures. The site of HSA binding by the liver is of great importance, and direct infusion of albumin into the hepatic portal vein is the most appropriate method of maintaining colloid pressure and cellular nutrient levels.

Keywords: human serum albumin; COVID-19 vulnerabilities; fluid therapy; albumin binding deficiency; lymphatic nutrient pump; colloid pressure; interstitial spaces; albumin infusion; hepatic portal vein

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1. Introduction

HSA is the main transporter of endogenous and exogenous ligands and the main component in regulating interstitial pressure. Although some drugs are known to be preferentially bound to prealbumin and gamma globulins, HSA comprises 50% of plasma protein content and regulates 80% of normal plasma colloidal pressure in the small capillaries and endothelial cells [1,2], as demonstrated in studies with iodinated albumin. Other nutrient binders can have a similar, though lesser, role.

HSA is a very large transport protein that binds to nutrient ligands in the intestine and liver, transporting ligands to the small capillaries and interstitial spaces, where HSA has a 7 h half-life. In our previous paper [3], we showed that HSA and other transport proteins increase the levels of nutrients available to cells and increase fluid reabsorption. Ligands attached to HSA are transported as a hydrated solid HSA–ligand complex. HSA nutrient ligands held in this manner are in equilibrium with the same nutrient ligands in solution, resulting in the plasma containing a concentration of nutrients many times that of the solution alone. The HSA–ligand complex then both transports the nutrients and increases the reabsorption of fluids from the interstitial spaces and cells. Molecules with similar molecular weights compete for dissolution in the plasma and lymph. Therefore, changes in the binding of HSA have consequences for every molecule in the plasma [2,4]. HSA also binds to COVID-19 virions and corresponding antibodies [3] as well as many of

the drugs, including those used to treat COVID-19 patients [3,5]. Most HSA remains within the cellular spaces, forming a 'pool' of HSA. Pool-HSA is an effective carrier permitting plasma to transport many more nutrients to the cells. HSA is produced, and the levels are maintained by pressure catalysis of precursors by the hepatocytes of the liver [1].

HSA is created by liver hepatocytes and rapidly excreted into the bloodstream, where it has a half-life of about 18 days. After 2 h, 90% of secreted albumin remains within the intravascular space. Serum albumin functions as a significant modulator of plasma oncotic pressure and as a transporter of endogenous and exogenous (virions and drugs) ligands. Albumin secretion is not controlled directly by the liver. Secretion is a direct result of a change in oncotic pressure in the hepatocytes of the liver; as albumin is responsible for up to 80% of oncotic pressure, a normative rate is maintained. Factors in increasing pressure in the hepatocytes can be cardiovascular and colloidal pressure [1,3].

Normal levels of HSA vary considerably between individuals according to physiology age and build (Figure 1). Hypoalbuminemia is defined as 3.5 g per decilitre from a normative value of 4.5, suggesting that a 20% drop from normal is sufficient to cause physiological damage for the average person. This corresponds to illness from hypoalbuminemia in half the population, concurrent with the 20% drop in HSA binding sites. For individuals in which albumin is impaired below 4.0 g/dL, such as over 50 s, or those with vulnerabilities, a small decrease in albumin-binding sites will precipitate stress in endothelia and localised pressure changes. The albumin molecule is many times smaller than the COVID-19 virion and more than one molecule of albumin may bind to a virion depleting available nutrient-albumin binding [1,3]. In addition, albumin carries ions such as Ca^{2+} and haem. Low in vitro albumin exhibits a coagulant action [6] and thromboembolic events [7].

The binding of HSA to nutrient ligands can be due to covalent bonds, hydrogen bonds, or van de Waals forces. For some ligands such as glucose, binding at low concentrations is by hydrogen bonding, and at higher concentration, glycation takes place, forming covalent bonds. In many cases, bonding is reversible either with or without enzymatic behaviour [8]. Albumin-binding permits more nutrients to be transported by the plasma by the formation of bonds, which then effectively 'entrap' nutrients by removing them from solution and allowing other similar-sized and charged molecules into the solution. Nutrient ligands are therefore transported as a part of the albumin complex, which exhibits discrete colloidal pressure according to hydration. Large nutrients attached to albumin exert a higher pressure than smaller waste molecules (Figure 2c).

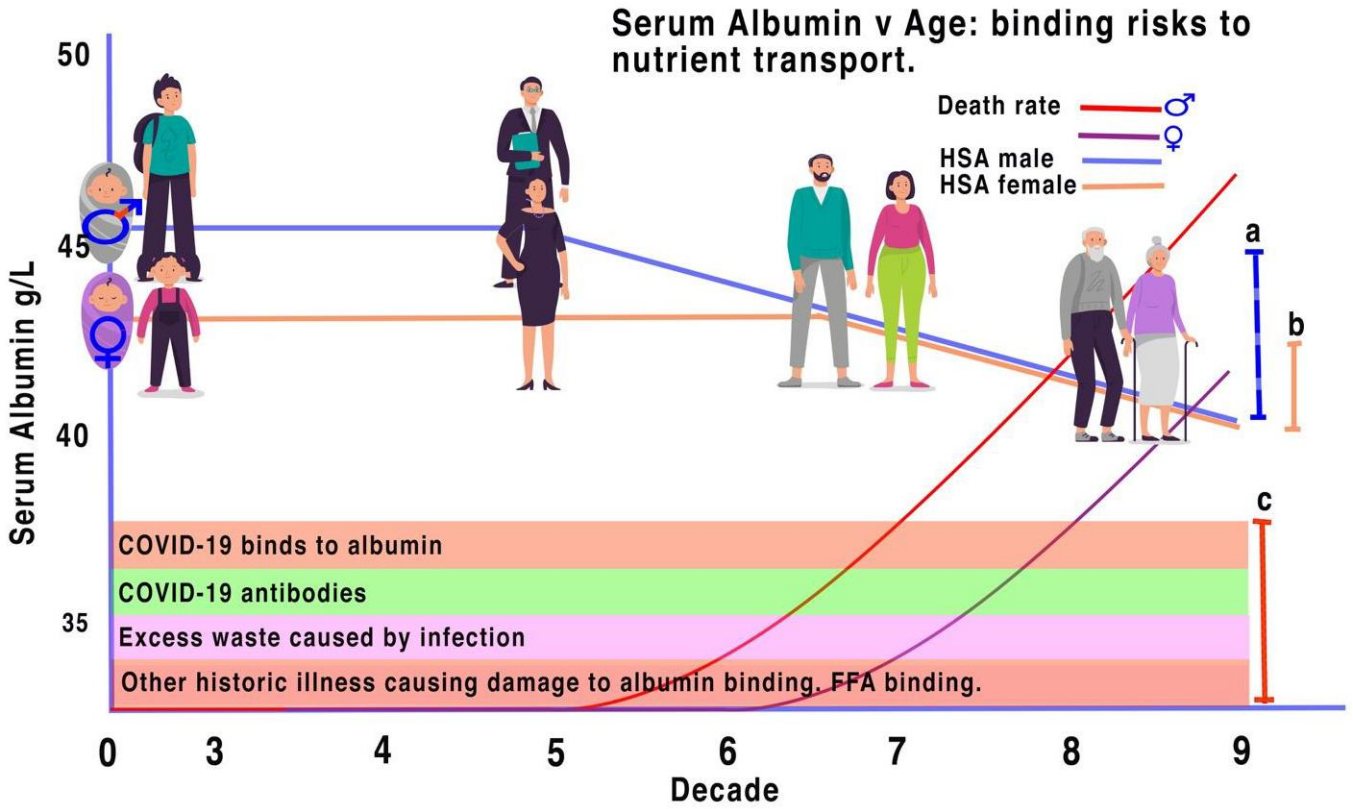

Figure 1. Illustrative profile match of albumin decrease with age and risk of COVID-19. Levels of albumin binding changes during ageing, making older males more vulnerable after 50 years and females after 65. Serum albumin levels of males (a) decrease with age earlier than those of females (b) (derived from [9]). SARS-CoV-2 virions, antibodies, excess waste, and factors from other illnesses reduce the tolerance of unbound albumin further (c). When the number of ligands caused by COVID-19 (c) exceeds that of either the male HSA binding tolerance (a) or the female HSA binding tolerance (b), the ability of HSA to transport nutrients is exhausted. The implication is that, as SARS-CoV-2 virions enter the system, they and the consequential antibodies and other created ligands block the natural ability of HSA to bind the correct nutrients, causing cellular stress and crisis in the systemic system and affecting all organs, leading to excess death rates in both males and females as illustrated (curves derived from data [10]). Human figures designed by Tartila/Freepik. Reprinted from [3] with permission under Creative Commons licence CC BY-NC.

2. Long COVID-19

HSA is known to act as a receptacle in protecting bacterial microbiomes [11]. A microbiome formation is possible between COVID-19 virions [12]. Due to the long half-life of HSA both in the interstitial spaces and the body, any bound COVID-19 virions, therefore, may be trapped for weeks or months. This entrapment of COVID-19 virions may be an explanation for long COVID-19 and recurrences of COVID-19 symptoms. HSA levels, therefore, maintain the healthy levels of nutrients to all organs of the body and control is by the liver.

Evidence That Low HSA Is Responsible for COVID-19 and Long-COVID-19 Vulnerabilities

There is now overwhelming evidence that a low level of human serum albumin (HSA) in patients is a predictor of vulnerabilities to COVID-19 and long COVID-19. Furthermore, HSA levels decline with age and are correlated with vulnerabilities to COVID-19 infection (Figure 1), as discussed in [3,5]. In Figure 2a, we illustrate the timing logistic of HSA production. HSA is bound to nutrient ligands in competition in the liver, which is provided with 20% of blood flow in a circulation that recharges every 2 min. The liver is a highly

complex organ that both produces HSA and modifies nutrient binding according to its own feedback. Control of HSA concentration and, thus, nutrient moderation takes place during each circulation, corresponding to a half-life of 7 h. HSA then passes to the heart and the lungs.

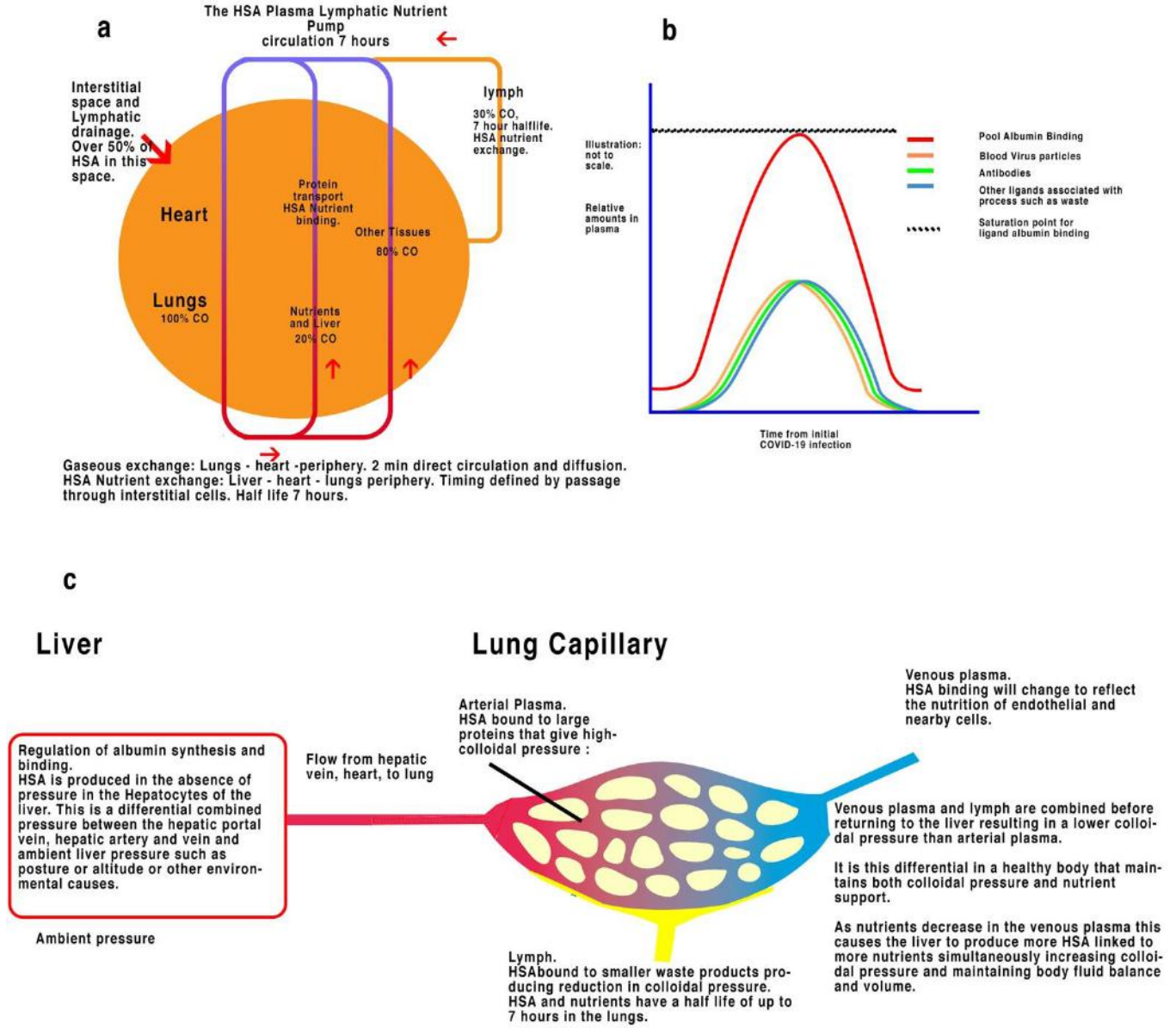

Figure 2. (**a**) An illustration of the HSA plasma lymphatic pump. HSA is charged with nutrient ligands for every 2 min circulation through the heart–lungs–liver circulation using 20% of cardiac output. The rest of the cardiac output travels to the periphery, where 30% passes into the interstitial cells and remains with a half-life of about 7 h depending upon organ and fluid flow. It is the albumin flowing through the interstitial fluid that facilitates nutrient support, colloidal pressure, and waste removal. (**b**) An illustration of the effect on albumin binding behaviour during infection, showing a decrease in binding potential. (**c**) An illustration of the colloidal pressure and nutrient changes due to albumin binding of nutrients and waste at different points in lung HSA Lymphatic Nutrient Pump.

Cellular nutrition is achieved through the circulation of HSA through the heart–lungs–lymph circulation, taking 7 h or longer in infection. Figure 2b is an illustration of an infection with COVID-19 showing a reduction in albumin binding as the blood virus particle waste, antibodies, and other ligands reduce albumin binding. As the disease increases in severity,

HSA binding is reduced by diminution of HSA and by the ligand binding caused by the disease. Figure 2c illustrates colloidal pressure decreasing across the capillaries due to the exchange of nutrients.

3. Vaccines Revisited

Just 14% of people in low-income countries have received at least one vaccine dose, compared with about 80% in high- and upper middle-income countries (WHO). Vaccines were discovered in the late 19th century, and their mechanisms were elucidated not long after. Although technical advances to preparations have been made over the last 150 years, their method of action remains as was for Pasteur and Jenner. It is important to briefly describe and repeat the mechanism of vaccines and their limitations in relation to the body's own immune system to place some context on their effectiveness to combat disease in vulnerable individuals. Vaccines are not medicines; they bear no relation to drugs that have defined physiological roles. A vaccine acts as a foreign body to encourage a body's own physiology to produce antibodies. The active molecules are not the vaccine but the corresponding antibodies produced by the response of the body to a foreign object (the vaccine). For the vaccine to be effective, the antibody must precisely match the foreign body (vaccine) and the vaccine must precisely represent the formation of the virus particle or other target. The vaccine itself has no direct effect in combatting the disease but works indirectly through the normal immune system of the body. Timing is critical as is the efficiency in manufacture when transcribing COVID-19 virion variants to a "keyed" vaccine and subsequently the body's effectiveness of transcribing the vaccine to "keyed" antibodies. Advances have been made to produce vaccines accurate enough to produce antibodies of varying specificity to the new COVID-19 variants. The available evidence suggests that vaccines may become less effective [13] over time.

The second process producing antibodies is performed by the body's own immune system, and its efficiency depends upon the physiological state and adaptations of the host. For most individuals, COVID-19 is asymptomatic. Therefore, in a healthy state, our bodies find no difficulty in eradicating COVID-19 virions. This paper is concerned with aligning vulnerabilities to the systemic mechanism of nutrient transport to cells in relation to albumin binding of COVID-19, and concomitant antibodies and waste and with describing the common factor albumin.

4. Generation of Vulnerabilities That Spread Systemically to Cause Complications

4.1. Albumin-Binding Deficiency

Figure 2b illustrates the passage of time during COVID-19 in relation to an infection and shows the rise in multiplying COVID-19 virions, antibodies, and waste products, any and all of which reduce the capacity of albumin for binding. As COVID-19 virions replicate, their concentration in the systemic system increases, followed by antibodies produced from immune response; these all bind to albumin. The immune response also creates waste ligands. The relative ascent of the curves is a variable of infection and immune responses. COVID-19 virion increase, and antibody immune response depends upon factors that affect immune response such as vaccination status or health issues. However, the timing always produces a decrease in albumin binding. All three types of ligands—virions, antibodies, and waste—compete for transport with the protein carrier system, the most abundant of which is HSA. As the levels of ligands increase the albumin-binding potential is reduced for the nutrient ligands such as glycolates that maintain the integrity of the endothelial capillaries [5,14]. As competition increases between different ligands and HSA binding sites in the plasma, interstitial fluids, and lymph, the intracellular concentration of ligands also changes. These changes in ligand-nutrient concentration are a result of the different binding opportunities afforded by ligands during capillary exchange when albumin binding becomes deficient and has implications for patients with COVID-19 vulnerabilities. This capillary exchange may also affect colloidal pressure. Albumin-binding deficiency can occur because of insufficient albumin and/or insufficient binding sites; both

are relevant to COVID-19 and long COVID-19. Albumin binding deficiency has been shown in chronic kidney disease [15]. Oxidative damage may also impair the binding properties of albumin. In advanced liver disease, a reduced binding capacity of albumin site II has been found mainly related to impaired liver function [16].

4.2. Common Damage to Epithelial Cells

In a recent publication [3], we discussed systemic septic shock and defined sepsis as the "systemic decrease in available albumin-protein binding sites for glycolates", leading to a decrease in the endothelial glycocalyx. The layer provides the integral support mechanisms for cellular adhesion, while a decrease will lead to cellular instability. Endothelial permeability appears quite early in the progress of aging. Aortas of 30-month-old rats had a two-fold increase in endothelial permeability to albumin compared with 10-month-old rats [17]. Endothelial cells lining blood vessels form a continuous layer that constrains proteins and blood elements to the vascular lumen. An increase in endothelial cell isometric tension (contraction) may disrupt the continuous endothelial barrier, leading to an increase in permeability and development of oedema, a hallmark of acute and chronic inflammation [18]. Recently, a comprehensive COVID-19 treatment protocol has been suggested involving the need to preserve the glycocalyx involving *N*-acetylcysteine (NAC) and other sulphur donors by optimising inorganic sulphate availability and, therefore, sulfation [19]. During COVID-19, any albumin-nutrient deficit may result in weakening of the endothelial cells, for example due to dissolution of the glycocalyx layer [5,14]. This leads to infection across barriers such as the gut and blood–brain barriers. The glycocalyx layer also becomes undersulphated in COVID-19. "The undersulphated glycocalyx may not only increase susceptibility to SARS-CoV-2 infection, but would also result in a hyperinflammatory response, vascular permeability, and shedding of the glycocalyx components, giving rise to a procoagulant and antifibrinolytic state and eventual multiple organ failure" [20]. Once the barrier function is compromised, large molecules such as albumin pass through, changing both pressure and nutrient support. The weakening of this layer changes cellular structural integrity, leading to secondary infections, loosening of intercellular adhesion, and direct changes to the nutritional medium of the cell and thrombosis. The effects of sepsis are, therefore, cellular in nature and are dependent on the type of cell and function not necessarily organ based. That many organs are affected concurrently indicates the common factor of albumin binding deficiency (ABD) and is linked to the lymph nutrient–albumin pump.

4.3. The Lymphatic System and Plasma–Lymph Nutrient–Albumin Pump

How is the albumin charged with nutrients? The lymphatic system maintains tissue interstitial pressure by collecting protein-rich fluid that is extracted from capillaries. The lymphatic system is also a critical component of the immune system (Figure 2a).

The lymphatic system is conventionally regarded as part of the immune system, in part because of the changes that take place during infection. A closer look reveals a system that can be considered being made of channels starting as the leakage of fluid from capillaries through endothelial cell gaps into interstitial spaces. This fluid initially resembling plasma and containing the full protein–ligand complements of plasma infuses the interstitial spaces around cells, providing membrane surface area access to cells. Flow is determined by the mechanical action of movement and muscle rather than from cardiac activity, with the lymph formed flowing back into the venous system and eventually the vena cava and heart. The circulation of albumin is complementary to the cardiac circulatory system to which it returns extracellular fluids [2]. Transport of nutrients in the blood, therefore, follows a separate circulatory pattern to that of the gaseous respiratory system, and rather than a few minutes, it may take hours or weeks for an albumin molecule to circulate in a normal healthy subject due to the half-life of albumin within the interstitial fluid (Figure 2a).

Nutrients enter through the stomach and are transferred via the hepatic portal vein (HPV) into the liver. Hepatocytes in the liver perform an enzymatic modulation of HPV and hepatic arterial plasma, responding to hormones such as insulin to maintain glycogen. The

liver can store and metabolise molecular structures. It can both metabolise and manufacture albumin according to pressure. Organ body fluid concentrations of nutrients and colloidal pressure are, therefore, moderated and controlled by the liver. Both released and circulating albumin bind to the moderated concentrations of molecular nutrients until equilibrium is formed by mixing. Nutrient bound albumin then passes through the heart to the lungs.

In the lungs, 60–80% of the nutrient–albumin complex passes into the interstitial spaces and remains there for many hours before returning to the vena cava through the lymph, the rest remaining in circulation. Only about 10% will be recharged by the liver. In the lungs, some nutrients on the albumin complex are exchanged for waste products because of metabolism. In healthy patients, the moderation of nutrients with albumin is assumed to be minimal; however, any illness or damage to the lungs will cause this equilibrium to change. In COVID-19-infected lungs, COVID-19 virion antibodies and waste replace nutrient ligands, changing both nutrients and colloidal pressure [21]. The aerated plasma is then pumped by the heart to the organs and periphery. Only about 10% of the lymph returns to the liver to be recharged with ligands. In a healthy subject, the continual mixing and rapid supply of re-bound albumin through the capillary circulation is enough to prevent albumin-binding deficiency. During COVID-19 infection in the lungs, there will be a rise in COVID-19 virions, corresponding antibodies, and detritus. In addition, secondary infections will have the additive effect of creating further antibodies.

4.4. Liver

The liver is the main control of nutrient ligands and waste in the body and the site of albumin synthesis [22]. The feedback process to evaluate nutrients and controlled supply is indirectly provided by the ligand–albumin complex. Albumin synthesis is directly linked to the reduction in pressure in hepatocytes caused by insufficient HPV blood pressure. Albumin provides 80% of oncotic pressure and the addition of albumin increases blood volume and pressure in the hepatocytes self-regulating overall blood volume and pressure and concurrent nutrients. A lack of nutrients in the periphery changes colloidal pressure by changing the binding of nutrients [21], metabolism, and hydration, thereby reducing the amount of albumin in the blood because of low lymph flow and metabolism of albumin. When nutrients are bound to albumin, they are removed from solution but maintain most of the hydrogen bonds responsible for their oncotic pressure. Bound albumin removes ligands from solution but retains the ligand's hydrophilic ability, increasing osmotic pressure. This causes low pressure in the hepatocytes and instigates the production of albumin. Oncotic pressure reduction will occur across the system, including on the hepatocytes, which produce more nutrient bound albumin.

4.5. Obesity

The most prevalent vulnerability to COVID-19 is obesity. Obesity is a worldwide major public health problem affecting many organs, including the heart, where it can cause heart disease, stroke, high blood pressure, diabetes, cancers, and dermatological complaints. Obesity has metabolic effects, such as causing hyperandrogenism and gout, which in turn are associated with cutaneous manifestations [23]. Dermatological manifestations of a systemic disease, such as gout, must have a systemic common factor.

Fatty acid concentrations in the plasma, interstitial fluids, and lymph have been shown to reflect the concentrations in adjacent fat cells. More fat in cells create a corresponding higher level of fatty acids in the interstitial spaces [3]. Fatty acids are transported by albumin, and greater obesity leads to a higher concentration of fatty acids in the blood and bound to albumin, causing binding deficiency. As fatty acids rise in the interstitial fluids, lymph, and plasma, more remain bound to albumin in circulation. This reduces the number of available binding sites on albumin for other nutrients to supply endothelial and cellular structures. A reduction in binding sites sufficient to affect nutrients eventually destabilises cell integrity.

4.6. Diabetes

Both insulin and glucose are transported by albumin as well as competing fatty acids. Restricting insulin access to albumin-binding sites in COVID-19 reduces the concentration of insulin concentration delivered to the liver with the subsequent elevation of glucose. This excess glucose may result in glycosylation of albumin further reducing binding sites. A reduction in albumin predicts type 2 diabetes [24]. This process is promoted by the presence of elevated blood glucose concentrations in diabetes and occurs with various proteins [25]. Glycated albumin also suppresses glucose-induced insulin secretion by impairing glucose metabolism in rats [26] and pancreatic β-cells dysfunction through autophagy [27].

Glycated albumin has a greater affinity for virions than albumin, and the ability of bacteria and viruses to surround themselves with serum proteins is a recognised immune evasion and pathogenic process [28]. SARS-CoV-2 spike binding protein binds to glycated serum albumin [28]. Long-term binding of virions in interstitial spaces would slow the flow and isolate COVID-19 virions shielded by albumin for many weeks and may be an explanation for Long-COVID-19.

4.7. Arthritic Pain

Biological activity regulation by protein post-translational modification (PTM) is critical for cell function, development, differentiation, and survival. Dysregulation of PTM proteins is present in various pathological conditions, including rheumatoid arthritis (RA) [29]. A decreased albumin/globulin ratio in RA patients significantly correlates with dyslipidemia and ARDs, implicating the albumin binding limits of fats concurrently changing [30].

4.8. Lungs

An unusual feature of the COVID-19 disease is microthrombosis and localised disruption of the osmotic potential with pulmonary microvascular dilation, a commonality in sepsis-induced ARDS [28]. In COVID-19, there is a greater risk of thrombosis [31] and coagulation [32]. Most patients are asymptomatic, with only a few patients severely affected. The resultant stagnation of HSA and ABD will reduce the levels of waste and distort the action of cellular structures, providing a possible mechanism for the "ground glass lung opacity", seen in COVID-19.

4.9. Heart

The risk of cardiovascular problems, such as a heart attack or stroke, remains high even many months after a SARS-CoV-2 infection clears up [33] and can affect even those with mild symptoms. The heart is a dynamic organ in continuous movement regulating pressure and flow of blood to the whole body; importantly, the movement of the heart also determines heart lymph flow, determining the amount of nutrients transported to essential heart cells. In disease, the first limiting factor is usually oxygen supply, where deprivation can produce stress within seconds; for medical practitioners, this is usually the first concern. Secondary to this, to maintain functional stability, the heart cells must be infused with nutrients. This occurs over a longer time-period, with albumin-charged nutrients lining the endothelial glycocalyx, protecting the stability of both capillary walls, and maintaining the correct supply of nutrients. This is dependent upon the albumin lymphatic pump over a much longer timescale, as heart movement and lymphatic flow become restricted and nutrients slowly cease to be delivered. As discussed above, the heart is secondary to the lungs; a change in nutrient metabolism due to lung disease, producing a deficit in nutrients over time, will inevitably lead to further degradation of the heart and its function.

4.10. The Blood Brain, Placental Barriers, and Albumin Transport in the Kidney

A common factor for the blood–brain barrier (BBB), the placental barrier (PB), and the kidney is that normal movement of albumin is restricted, and in each case, albumin is controlled by clathrin enabled endocytosis [34,35]. Infection with COVID-19 leads to a

reduction in albumin binding sites, including that used by clathrin to initiate endocytosis of the albumin–nutrient complex. This blocks the albumin from entering the cell and passing the barrier in each case.

4.11. The Central Nervous System and the Blood–Brain Barrier (BBB)

Severe COVID-19 and long COVID19 are both associated with cognitive defects [36]. In healthy subjects, both nutrients and pressure are kept stable within the cerebral spinal fluid (CSF) by the action of the blood–brain barrier, which stabilises and regulates albumin, intercranial pressure, and bound nutrients. Both pressure and nutrient support are therefore maintained within controlled limits within the CSF in a separate environment to the cardiac circulation. In the CSF, where 95% of amyloid b is bound to albumin [37], any decrease in binding levels will have a direct effect on amyloid beta (Aβ) concentration, potentially increasing plaque formation [38]. Studies have shown that the possibility that patients with COVID-19-associated neurological syndromes exhibit impaired amyloid processing [39]. There is therefore evidence of a connection between neurological damage due to plaque formation, with a direct link to the control of Aβ by albumin and albumin binding levels [37].

During initial COVID-19 systemic infection, COVID-19 virions interfere with entry of a proportion of the albumin by occupying the binding site for clathrin. This leads to gradual nutrient deficit within the CNS. There will also be weakening of the capillary walls due to lack of glycolates [2], thrombosis [40], and disturbances of synapse connectivity as transmitter vesicles decay. As the disease progresses the blood–brain barrier becomes weaker, and rupture may ensue, allowing larger bacteria, in addition to viruses, to enter from the systemic system leading to meningitis. There may, therefore, be more than one action occurring during COVID-19 infection in the brain in regard to albumin:

(I) Virions attached to HSA may affect the transport of vital nutrients across the BBB. This nutrient deficit will depend upon the state of has-binding deficiency. This level of binding deficiency will alter the levels of transmitter and affect transmission of action potential affecting cognition.

(II) Depletion of nutrients will also affect the capillaries of the brain, for example, the endothelial glycocalyx layer (EGL) already described [3,5]. A reduction in the EGL will eventually cause leakage, thrombosis [40], and rupture. Rupture may promote secondary infection, leading to symptoms of meningitis [41].

4.12. Kidney

Pathology of COVID-19 in the kidney indicates symptoms of nephrotic syndrome, numerous glomerulonephritides, microscopic polyangiitis vasculitis and collapsing glomerulopathy, and thrombotic microangiopathies, such as atypical haemolytic uremic syndrome (aHUS) [42].

In healthy individuals, there is minimum albumin loss from the kidneys and any albumin is reabsorbed by the peritubular capillaries by phagocytosis [43,44]. The glomerulus, the filtering unit of the kidney, is a unique bundle of capillaries lined by delicate fenestrated endothelia [45]. A large percentage of COVID-19 affected patients present with acute kidney injury (AKI); most cases of CoV-AKI are driven by a form that can cause impairment in tubular reabsorption of filtered proteins [46]. Reabsorption of albumin is usually by clathrin-mediated endocytosis, as described above. This necessitates the binding of albumin to clathrin. Any ligand that competes with clathrin will change this equilibrium and permit albumin to pass into the urine. This also correlates with evidence that urinary excretion of uric acid is negatively associated with albuminuria in patients with chronic kidney disease [47]. The association between albuminuria and serum uric acid may not be interrelated via renal handling of uric acid [47] but by the levels of albumin-binding available [47].

4.13. Pregnancy

Previously [48], we noted that albumin is entirely metabolised by the foetus and is not therefore circulated by the liver. Pregnancy therefore removes bound albumin–nutrient complexes for the metabolism of the foetus, leaving a deficit that may be one cause of the adverse symptoms in preeclampsia [48]. A lack of albumin due to metabolism by the foetus is a plausible explanation for the stresses some pregnant women have experienced in the third trimester. For the same reason, in COVID-19, both the foetus and mother may experience reduced albumin-binding caused by both the permanent exclusion of returning albumin from mother to foetus and COVID-19 disease.

4.14. Skin: Distribution of Albumin in the Adult and Child and Infant Body

The human foetus metabolises albumin passed from the mother in the form of the albumin–nutrient complex. In the young child, albumin is concentrated in the periphery and the muscle and skin; this may be caused by children having a larger surface area to volume ratio. In the adult, the lungs and organs contain relatively larger proportions. There are great variations between individuals and ages. There have been many reported instances of dermatologically significant issues [49], including, thrombosis, chilblains [40,49–52], mucocutaneous disease [53], purpura [54], and rashes. The frequency and timing of cutaneous manifestations of COVID-19 are difficult to ascertain; also unclear is the association of certain skin manifestations with the illness severity. Moreover, it cannot be excluded that, in some patients, the observed skin findings may represent cutaneous reactions to the treatments used for COVID-19.

Obese COVID-19 patients have a high occurrence of dermatological problems. Increased body mass index affects skin physiology, skin barrier, collagen structure, and wound healing. Obesity also affects sebaceous and sweat glands and causes circulatory and lymphatic changes. Furthermore, obesity is associated with an increased incidence of bacterial and Candida skin infections, as well as onychomycosis; inflammatory skin diseases; and chronic dermatoses such as hidradenitis suppurativa, psoriasis, and rosacea. Obesity is also related to rare skin conditions [23]. Obese children have a higher prevalence of skin lesions than normal weight children [55].

In COVID-19 infection, the dermatological signs are diverse and the timing is irregular. There is a greater resting pool of albumin during COVID-19, remaining in the interstitial spaces for longer; a lack of movement during illness reduces the activity of the HSALNP HSA lymphatic nutrient pump, isolating the stagnating albumin and causing albumin binding deficiency in associated areas depending upon flow. For the skin of a child, therefore, dermatological nutrients bound albumin and, therefore, nutrients will be decreased in relation to the percentage of albumin flow, with changes in colloidal pressure. The timing of this is dependent upon the nutrient-bound albumin flow into the interstitial spaces. The pooling of albumin may be provoking dermatological reactions independently from the COVID-19 infection sites. Dermatological conditions, therefore, will vary according to localised albumin pooling, timing, and binding deficiency. A lack of available albumin binding may therefore instigate systemic nutrient deficiency, leading to symptoms of multisystem inflammatory disease, where apart from obesity (25.3%), comorbidities are rare [56].

Hypercortisolaemia is a condition involving prolonged excess serum levels of cortisol that can develop as a result of disregulatory abnormalities in the hypothalamic-pituitary–adrenal axis or from exogenous-source steroids. Hypercortisolaemia induces a state of immunocompromise that predisposes the patient to various bacterial, viral, fungal, and parasitic infections [57]. Low serum albumin levels in patients with ischemic stroke are associated with higher serum cortisol levels and predisposes to hypercortisolaemia [58]. High serum total cortisol concentrations are associated with high mortality from COVID-19 [59].

Inflammatory markers and acute phase reactants (“markers”) are also associated with COVID-19 infection and may be able to predict disease severity [37,60,61]. Negative acute phase reactants are downregulated, and their concentrations decrease during inflammation.

Positive acute phase reactants include procalcitonin, C-reactive protein, ferritin, fibrinogen, hepcidin, and serum amyloid A. Negative acute phase reactants include albumin, prealbumin, transferrin, retinol-binding protein, and antithrombin. A reduction in albumin binding will have a concurrent effect on marker concentration.

4.15. Fluid Therapy

The use of fluid therapy is ubiquitous in medicine, with all medical staff, doctors, nurses, and many ancillary staff trained in infusion techniques. "Intravenous fluid therapy is one of the most common interventions in acutely ill patients. Each day, over 20% of patients in intensive care units (ICUs) receive intravenous fluid resuscitation, and more than 30% receive fluid resuscitation during their first day in the ICU. Virtually all hospitalized patients receive intravenous fluid to maintain hydration and as diluents for drug administration. Until recently, the amount and type of fluids administered were based on a theory described over 100 years ago, much of which is inconsistent with current physiological data and emerging knowledge. Despite their widespread use, various fluids for intravenous administration have entered clinical practice without a robust evaluation of their safety and efficacy. The belief that dehydration and hypovolaemia can cause or worsen kidney and other vital organ injury has resulted in liberal approaches to fluid therapy and the view that fluid overload and tissue oedema are 'normal' during critical illness; this is quite possibly harming patients. Increasing evidence indicates that restrictive fluid strategies might improve outcomes." [62]. Attempts at albumin infusion have been inconclusive [63,64], but there is ongoing discussion of its merits [62,65].

5. Discussion

Nutrient support in cells is an essential method for maintaining both physiological stasis and immune response. In a healthy body, albumin is charged with nutrients in the liver and exhibits discrete osmotic pressure according to nutrient bonding. Pumped by the heart in circulation and muscle in the lymph, organs divert at least half of the nutrient bound albumin into the interstitial spaces and lymph; the rest flows through the capillaries and is a part of cardiovascular lung systemic circulation. The diverted albumin that passes into the interstitial spaces exchanges nutrients and binds to waste products. This changes both the nutrient bound to albumin and the colloid osmotic pressure exerted by the changing of ligands on the albumin molecule. Albumin then passes back via the lymph into the systemic circulation. Less than 10% of albumin will be recharged with nutrients on each circulation. A decrease in mean albumin levels by 10% is sufficient to create symptoms of hypoalbuminemia. For individuals aged 50+, albumin level decreases significantly (see Figure 1). Any excess fatty acids such as in obesity further reduces albumin binding.

5.1. Physiology

The liver is the central control organ for cellular nutrition, colloidal pressure, and waste removal by a dual circulation. The capillary circulation and the lymphatic circulation of HSA, which it feeds, are directly linked to the pressure of the liver. Changes to arterial or venous pressure, or ambient pressure will affect the stability of HSA production, binding, and nutrient and colloidal support. The production of albumin is affected by all pressure changes to the body, including outside ambient pressure (Figure 2c). This may account for the increase in HSA measured during ascension to altitude [66] and may have importance for acclimatisation.

5.2. Why Is Present Fluid Therapy Inappropriate in COVID-19?

The present purpose of fluid therapy (FT) is to correct colloidal pressures in the cellular structures. Saline provides a change in pressure but dilutes nutrient ligands with unpredictable results. HSA's effect on oncotic pressure is well known and is partially down to the nutrients bound. Changes to colloidal pressure by HSA FT can therefore only be predicted knowing the correct relevant ligand–albumin binding for each organ (pulmonary

artery, aorta, and vena cava will have differing albumin bindings with differences enhanced as HSA passes into cellular spaces). In a healthy body, this difference between ligand–albumin binding in the lungs and that delivered to the periphery will be minimal. However, in COVID-19, damage to the lungs causes HSA to be restricted, thus reducing the amount of nutrients with resultant immune reactions. Furthermore, contemporary FT infusion is given via a peripheral vein such as the brachial vein (Figure 3). The lungs receive 100% cardiac output, but the liver only receives about 15% of cardiac output; 85% of HSA infused to the vena cava through the periphery will not be charged with nutrients during circulation to the liver and will not be providing either the correct nutrients or oncotic pressure to the cells and interstitial spaces. HSA has a half-life in intracellular spaces of about 17 h so that any immediate replenishment will be compromised, and nutrient deficiency will take place during this time. Furthermore, the serum albumin content determined by peripheral vein analysis may not represent the content of pool albumin in the interstitial spaces, especially during illness and poor blood flow.

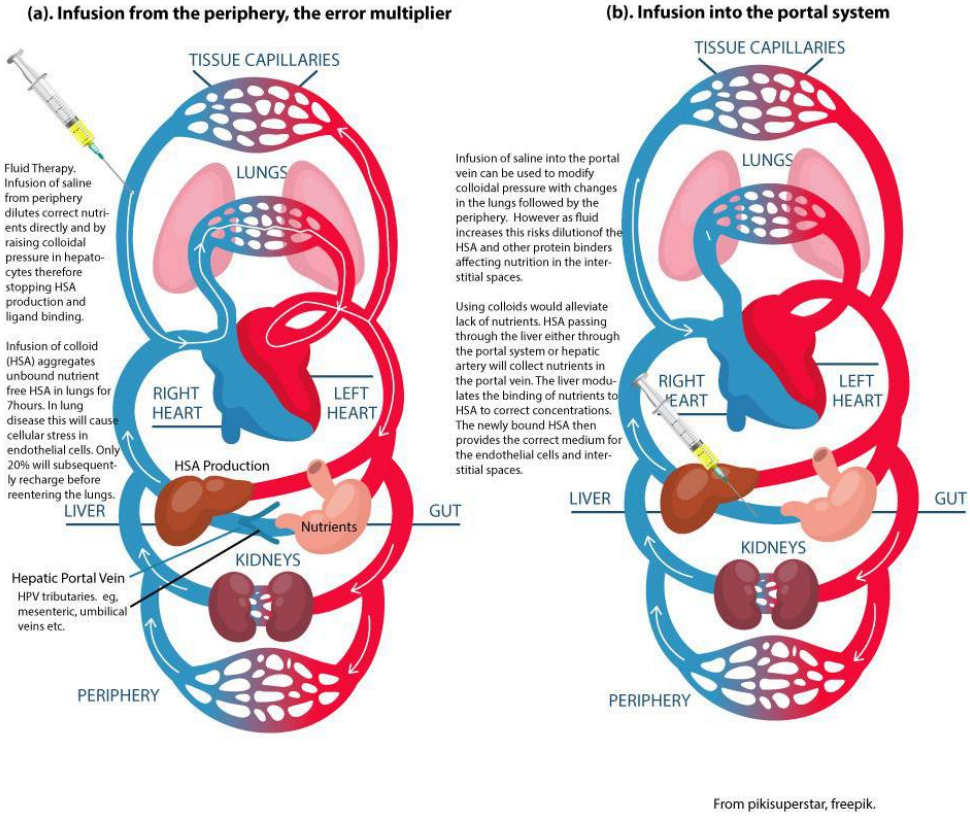

Figure 3. Illustration of the liver and application of fluid therapy. The bulk HSA added to the periphery does not enter the liver to be appropriately charged with nutrients but remains in the interstitial fluid and lymph for many hours, leading to nutrient deprivation and sepsis. (**a**) Inappropriate application of fluid therapy to periphery leading to nutrient and pressure deformations in the lungs. (**b**) Appropriate application of fluid therapy to portal vein providing correct pressure and nutrients to all target organs. Circulation modified from pikisuperstar/Freepik with permission under Creative Commons licence CC BY-NC.

5.3. Saline

Saline initially rehydrates the cellular structures; however, this reduces the levels of nutrients in the plasma and intracellular spaces. Diluting available albumin may also result in waste deposits not being removed. The addition of saline also results in a rise in pressure in the hepatocytes, which will reduce albumin production, binding to nutrients, and subsequent nutrient output into the systemic system. Between individuals, there is great variation in the levels used natively to regulate pressure and nutrients. Saline FT can,

therefore, only safely be used to rehydrate a patient to a hydrated state when it is known that nutrient support is maintained, and organ damage is minimal. Continual use of saline deprives cellular structures of nutrients.

5.4. HSA Infusion

The infusion of colloids into the periphery was described in Figure 3. Manufactured HSA preparations contain no nutrients, and colloidal pressure will differ from that of HSA in situ. Infusions of HSA into the periphery flow through the heart and into the lungs, where more than 50% rests in the interstitial spaces for many hours before re-joining the venous flow to the heart. From the heart, only 10% of the total albumin in the plasma will flow to the liver, with the remainder passing to the peripheral capillaries to be divided again between the capillary flow and the interstitial spaces. Any further HSA addition will lead to an increasing nutrient deficit within the extracellular spaces (Figure 2a).

Where the main concern is the liver, for example to treat cirrhosis, albumin infusion into the periphery has been shown to be highly beneficial [67]. We suggest that its success is because liver function is the main therapeutic target organ. The liver has the ability to bind the albumin or, at higher infusion rates, to metabolise HSA to provide further metabolites. At low rates of infusion, the liver will recover, but there will be little or no effect on other systemic organs unless they have independent vulnerabilities; at higher rates or longer application, both nutrition and pressure in the interstitial spaces will change, causing depletion of nutrients and stress, limiting this method. Higher rates of infusion to produce benefit to the periphery and CNS is probably only achievable directly through infusion to the liver via the portal vein, therefore producing nutrient bound HSA at the correct colloidal pressure.

Albumin-binding deficiency (ABD) occurs when insufficient binding sites remain on the albumin molecule to sufficient supply cellular and intracellular structures with the required nutrients. This may occur with any ligand that binds to albumin or other molecules in the plasma. This occurs due to insufficient albumin and/or insufficient binding sites. COVID-19 virions, antibodies, and vaccines are carried on albumin. A lack of albumin causes energy depletion from glucose, mitochondria failure, and cell death, as shown in Table 1.

Table 1. Key deductions on albumin binding and its appropriate therapy.

There is repeated evidence of a connection between hypoalbuminemia and COVID-19 for each symptom of and vulnerability to COVID-19. HSA binding deficiency is a common factor.
There is evidence that raising HSA concentration in the liver may alleviate some of the vulnerabilities to COVID-19 by reducing any HSA binding deficiencies.
A mechanism for albumin involvement in long COVID-19 also exists and could be removed by appropriate HSA therapy, given that the liver precisely modulates nutrients in the plasma and maintains HSA levels.
Present fluid therapy, either saline or colloid, applied to a peripheral vein, results in a destabilisation of nutrient transport, leading to nutrient deficits in cells and cellular components because of albumin-binding deficiency.

Cellular nutrient support is dependent upon the flow of nutrients from the liver and must be adequately supplied from the hepatic portal vein from the stomach. Molecules compete for dissolution and relative concentration in fluids irrespective of protein binding so that most molecules carried in the blood are affected by albumin binding. Nutrient concentrations in the cells are defined by the levels of nutrients' capability to bind to albumin, which therefore affects other molecules of similar size and charge. Because albumin is the largest carrier of ligands in the blood and defines and regulates 80% of oncotic pressure, it is the main determinant of nutrient balance to the cells.

Although this review was created for an understanding of COVID-19 and long COVID-19, the theoretical details may be applied to many different abnormalities, each of which is

linked through albumin (HSA) binding mechanisms and their deterioration, where HSA binding levels are a common factor. Any disease or injury that decreases the levels of albumin binding such as secondary infections will also increase the risk of albumin binding deficiency and sepsis.

6. Conclusions

The appropriate method of fluid therapy is to infuse albumin to the liver directly through the hepatic portal vein. Both nutrient support for the cells and oncotic pressure are regulated by the liver by HSA concentration. Long-term saline or peripheral albumin infusion results in degradation of nutrients to the periphery.

COVID-19 attacks albumin binding and charging of nutrients by reducing blood and lymph flow; by causing excretion of albumin through the kidneys; by degrading normal liver function; and by decreasing the binding potential of HSA caused by excess COVID-19 virions, antibodies, and waste.

We have provided evidence that albumin binding deficit may be responsible for COVID-19 and long COVID-19. The obvious solution is to raise albumin flow to the liver to raise nutrients. The liver is a fast and adaptable moderator of nutrients that can supply itself from body stores almost without limitation.

Infusing albumin directly will lead to a fall in albumin creation by the liver and a possible reversal into metabolism of albumin to provide other nutrients, which will benefit the liver. Infusion to the liver also binds albumin to the correct nutrients for the systemic system and periphery within healthy limits; liver limitations are few. As the liver is the centre of control, unlike other remedies, the amount of albumin entering will be proportionate to the amount of nutrient-bound albumin exiting. Infusion into the liver should allow for proportional control over both colloidal pressure and nutrients.

Albumin must therefore be administered directly to the liver via the portal vein. There are tributaries to the HPV, such as the mesenteric and umbilical veins, that are only a few cm deep and could be ultrasound guided cannulated.

The concentrations of all systemic ligands transported in the blood including all drugs are affected by albumin binding, and there has been little consideration of the interactions between ligands and transporters.

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Abbreviations

HSA, human serum albumin; ABD, albumin binding deficiency; HSALNP, HSA lymphatic nutrient pump; LHLL, liver heart lungs liver; Ligand, any molecule capable of binding to albumin both nutrient and waste; HPV, hepatic portal vein.

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6.5 The HSA Lymphatic Nutrient Pump (HSALNP) and its critical and central importance to Health.

Andrew S Johnson and William Winlow. **"The HSA Lymphatic Nutrient Pump (HSALNP) and its Critical and Central Importance to Health".** EC Pharmacology and Toxicology 10.12 (2022):32-34 .

As a simple example: posture, climate, and ambient pressure all contribute to the ability of the body to maintain cardiac return to the heart largely by means of the Frank-Starling mechanism. Cardiac output maintains both gaseous exchange and nutritional support through the HSALNP and both are critical in maintaining health. This cardio-respiratory circulation is not secondary as it is as essential as gaseous supply – and more critical when considering ill, obese, or elderly individuals. Without a constancy of fluid volume and composition, the Frank-Starling mechanism operates inefficiently and failure results. The HSALNP defines the operating levels of the cardiac system over a longer time-period and the health of respective tissue and cellular structures according to HSA availability.

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EC PHARMACOLOGY AND TOXICOLOGY

Mini Review

The HSA Lymphatic Nutrient Pump (HSALNP) and its Critical and Central Importance to Health

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Abstract

The effectiveness of fluid therapy should be increased by infusion of human serum albumin (HSA) direct to the liver. HSA generation by the liver is the only factor available and capable of controlling whole body fluid volume levels and distribution. The remaining blood content blood cells, platelets, proteins are all controlled by other secondary mechanisms and follow the levels of albumin. Critical nutrients bind to HSA when HSA is made available by the liver. The HSA lymphatic pump circulates albumin through the interstitial spaces and lymph with a half-life of many hours. HSA levels may therefore have the ultimate control over all blood functions in healthy individuals and thus in the changes to nutrients and hormones transferred by the blood to the small cellular spaces. In the case of infected patients HSA binding deficiency may cause cellular deterioration and systemic sepsis. Infusing HSA to the liver should correct nutritional and colloidal pressure abnormalities in affected tissues.

Keywords: *HSA Lymphatic Nutrient Pump (HSALNP); Human Serum Albumin (HSA); COVID-19*

Introduction

Multiple variants of COVID-19 have led to an increasing number of vaccines that have minimised reinfection levels but have little chance of eliminating COVID-19. Sustained COVID-19 infections encourage new variants some of which may be dangerous leaving pharmaceutical companies playing vaccine catch-up. Vaccines do not affect the virus directly but act to stimulate the body to produce antibodies.

A more pharmacological solution is to attenuate the body's own immune response by decreasing any known limiting factors to cell health, this must include systemically transported cell nutrients and hormones. We have shown that albumin binding of nutrients by the liver is one such limitation. Many people are asymptomatic or unaffected by COVID-19 and we have shown a common factor is the level of albumin available for binding and transporting essential nutrients. Cell health is a function of the surrounding medium, the nutrients in this medium are determined by human serum albumin (HSA) levels. Furthermore, albumin binding deficiency (ABD) is implicated in all vulnerabilities to COVID-19, e.g. age, obesity, nutrition.

Human serum albumin lymphatic nutrient pump

The HSA lymphatic nutrient pump (HSALNP) [1] is critical as it defines the ability of the body to adjust to both posture, climate and pressure and its control is almost completely independent of the circulation of gaseous exchange, at the same time it controls resting cardiac pressure and cardiac output. It maintains the steady state of the cardiovascular system, immune system, cellular activity and responses to all administered drugs.

There is overwhelming evidence [1] that the circulation is not a singular concept as defined in conventional textbook physiology. There are quite clearly two or more circulations one for gaseous exchange (taking minutes) and another not controlled by the cardiac system, for nutrient distribution and waste removal (taking hours/days/weeks): this is controlled by combined colloidal pressure and the HSALNP from the liver and powered by body movement. Nutrients are maintained by the binding of HSA from all over the body but especially the liver, which is able to both produce and bind nutrients in 'real-time' without exhaustion, maintaining a consistent concentration of nutrients when the volumes of whole-body fluid (plasma, interstitial fluid lymph) change. These HSA-bound nutrients which critically circulate by posture, movement through the capillaries, interstitial fluid and lymph provide almost all nutrients to the organs according to individual physiology. The HSALNP forms a secondary circulation independent of the cardiac circulation which provides continuous monitoring of nutrient and waste status (nutrient bound HSA has higher colloidal pressure than waste bound) forming a natural homeostasis of nutrients, oncotic pressure in all organs individually and whole-body fluid composition. It is this circulation occurring over hours/days/weeks which forms the basis of acclimatisation due to pressure, altitude or depth.

As a simple example: posture, climate, and ambient pressure all contribute to the ability of the body to maintain cardiac return to the heart largely by means of the Frank-Starling mechanism. Cardiac output maintains both gaseous exchange and nutritional support through the HSALNP and both are critical in maintaining health. This cardio-respiratory circulation is not secondary as it is as essential as gaseous supply – and more critical when considering ill, obese or elderly individuals. Without a constancy of fluid volume and composition, the Frank-Starling mechanism operates inefficiently and failure results. The HSALNP defines the operating levels of the cardiac system over a longer time-period and the health of respective tissue and cellular structures according to HSA availability.

Current fluid therapy protocols

The present applied protocols for Fluid therapy involving saline are dangerously uninformed, being based upon historical theory from the first world war without scientific merit, and should never be used longer than for immediate rehydration of the primary cardiac circulation. The only logical remedy is to add HSA to the liver directly. We predict this will raise oncotic pressure and nutrients in appropriate concentrations concurrently reducing and removing many, or all the symptoms, as previously described [2]. At present this is not the case and we believe many unnecessary lives are lost each day and many condemned to suffer due to the absence of an adequate understanding of integrative physiology.

A more appropriate fluid therapy protocol

Here we propose that directly adding albumin to the liver via the hepatic portal vein to be charged with nutrients is the most appropriate protocol for fluid therapy. Peripheral infusion of both saline and colloid results in inappropriate nutrients and colloidal pressure. Infusing HSA direct to the liver should produce concomitant increase of bound HSA nutrients simultaneous with HSA concentration rise leading to a greater normalised whole blood volume, pressure and nutrients aligned to cellular efficiency. We consider that infusing saline peripherally is almost always contra-indicated in almost all cases where direct infusion of HSA to the liver is possible.

Conclusion

There is a discontinuity between the science and mechanisms of medicine and practical application where this subject has been relegated to history inappropriately and without logic. All clinicians administering fluid therapy should be required to understand this highly complex but critical system as it is the central mechanism that defines the steady state health of the immune system, cellular integrity and whole-body fitness, particularly as albumin binding deficiency is also implicated in sepsis [3].

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7. *Discussion*

7.1 Neuroscience

My research into how the brain computes has had far reaching consequences and has changed our understanding of computation in the brain. The brain is not a solid structure and nerves exhibit plasticity and changing connections in neural network circuitry. I started with the action potential, demonstrating its weakness for computation, and then forming a model the APPULSE. I showed that computation must occur between colliding thresholds and not spikes. The APPULSE is a combined mechanical soliton action potential whose speed is defined by the soliton. It is the speed of the APPULSE that allows frequencies to compute through phase ternary computation that accounts for computation in the retina. What we see in the retina is that this phase ternary computation maps object recognition in the optic nerve so that it can be understood as objects in the visual cortex. In our most recent work, we concentrate on the visual cortex and how these objects are used to learn.

In the anecdote of the two-year-old future tennis pro hitting a ball for the first time. They will go on to hit the ball many times and sometimes miss, but with every hit they perfect their moves. In the future grand-slam each and every volley is enveloped in their past providing the experience for perfection back to the two-year-old. Similarly, each and every volley is recorded for future use. In the same way we are a product of our environment. Our brain, containing 90 billion neurons, is not and cannot be completely defined by our genes and must, in some ways, be randomly formed, depending on our experience, particularly in early life. The world around us might superficially appear philosophically determinant, with maths dominating action-reaction logic. However, the sentient, human or other, are time travellers not taking their reaction just from the present but always referring to the past. Sentience is formed from our knowledge of the past and humans with the development of speech, and now other types of communication, form decisions on not just their own past, as animals, but also by communicating others' pasts. It follows that sentience is the formation of perception based upon previous knowledge, direct or indirect. It is the storing and mechanism of this knowledge in memory that allows us to learn simultaneous to activity because as in the tennis pro, each action is a learning experience. This does not of course change our philosophical knowledge of the hard question of individuality and consciousness, although we have arrived closer to being able to predict how perception and memory works, we are still far from separating subjective individuality.

7.2 Albumin (HSA) and COVID-19

Although my work concentrated on the epidemic and why vulnerabilities exist it is the overall discovery that albumin (HSA) is the overall determinant of whole-body fluids that I think is the most important. As albumin levels rise in the body then all other components rise concomitantly, blood and colloidal pressure, blood cells, nutrients, etc. This suggests that exerting a level of control over albumin to the liver will allow clinicians to determine the correct nutrients and contents of the blood. This is the most important factor in systemic illness, each cell is bathed in fluid which originates from the control of the liver. If the levels of fluid and nutrients can be tamed this will be of great use to clinicians in every subject.

7.3 Time

Time is an essential element of all my studies a common factor both in neuroscience and Albumin research. In the brain there is no clock, and synchronisation is applied through phase dependant action potential quanta (APPULSE).

In neuroscience I have shown that the temporal element of the action potential defines computation in the brain where the timing along neurons is critical. Computation is by temporal annulation of phase dependant action potential acting as quanta, timing is performed relatively by frequency.

COVID-19 is itself temporally dependant and my theories as to albumin depend upon the timing between infection and the body's response. If albumin binding is sufficient during infection most cells will be stable and the immune system will speedily react to annul the virus. If albumin binding is insufficient the virus and concurrent damage will outpace the immune systems' ability to prevent further injury resulting in a build-up of ligands in the systemic system producing sepsis.

8. Final Comment

In my next paper I will discuss how phase ternary computation is decoded by the visual cortex into image memory units. This field is ongoing with considerable discussion. Phase ternary computation occurs when action potentials are annulled due to the membrane refractory period having been activated by a preceding action potential. In a network such as the retina this results in computation by frequency rather than conventional computers which compute in set time. The APPulse and phase computation also have implications for artificial intelligence as this is a non-Turing system.

In addition, our work stemming from brain nutrition and COVID-19 has widened to where the story of HSA, its production, control, and distribution have all led to a central thesis that HSA is the central control element for cellular health.

Whole body fluid levels control the efficiency of the heart and lungs affecting both the cardiovascular and pulmonary system almost instantaneously. When fluid levels rise the binding of HSA promotes higher nutrients and colloidal pressure increasing flow. Sustained growth, illness and posture are forms of acclimatisation and must all be compensated for by a change in whole body fluid volume for the cardiovascular system to remain stable. Our hypothesis is that levels of fluid are maintained by albumin controlled by pressure variations in the hepatic portal vein, the level of albumin then in turn controls the levels of nutrients, colloidal pressure, and the medium surrounding cells. This provides evidence that direct infusion of HSA to the liver will allow finite control over nutrients, red blood cell count, colloidal pressure in individual cellular structures. Sustained delivery of correct medium to cell structures should alleviate systemic vulnerabilities to the diseases and illness covered in our papers.

My thesis started as an investigation into sentience and computation in the brain then diversified as a need to understand the effects of cellular nutrition on the brain into how stability and recovery during disease disrupts all cellular nutrition. A self-regulating liver mechanism (HSALNP) was

discovered involving albumin transport that explains whole body fluid distribution and fluid component levels in many organs.

9. Citations and Impact.

Statistics on the publisher's site and ResearchGate show we have been cited many times, with many thousands of readers for each paper. The impact of my COVID-19 work has been intense with communication with others in the same work. My work is continuing in both domains as can be seen from the many citations and interest.

10. All Thesis Publications

- Andrew S Johnson and William Winlow. **"The HSA Lymphatic Nutrient Pump (HSALNP) and its Critical and Central Importance to Health". EC Pharmacology and Toxicology 10.12 (2022):**
- Johnson AS, Polese G, Johnson M and Winlow W. **Appropriate Human Serum Albumin Fluid Therapy and the Alleviation ofCOVID-19 Vulnerabilities: An Explanation of the HSA Lymphatic Nutrient Pump**. COVID 2022, 2, 1379–1395. https://doi.org/10.3390/covid2100099
- Johnson, AS, Winlow, W. **Pre-Eclampsia, Hypoalbuminaemia and Albumin Therapy**. *Eur. J. Biomed. Pharm. Sci. 2021, 8, 75–78.*
- Johnson AS, & Winlow W. (2021). ***COVID-19 vulnerabilities are intensified by declining human serum albumin levels.*** *Experimental Physiology. 2021;1–9. DOI: 10.1113/EP089703*
- William Winlow and Andrew S. Johnson. **Nerve Impulses Have Three Interdependent Functions: Communication, Modulation, and Computation**. Bioelectricity.Sep 2021.161-170.http://doi.org/10.1089/bioe.2021.0001
- *Johnson AS and Winlow W (2021)* ***Does the Brain Function as a Quantum Phase Computer Using Phase Ternary Computation?*** *Front. Physiol. 12:572041. Doi: 10.3389/fphys.2021.572041.*
- *Johnson AS, Fatemi R and Winlow W (2020)* ***SARS-CoV-2 Bound Human Serum Albumin and Systemic Septic Shock****. Front. Cardiovasc. Med. 7:153. doi: 10.3389/fcvm.2020.00153*
- ***The role of hemoglobinopathy in COVID-19 pathology.*** *Fatemi R, Johnson A and Winlow W. Eur. J. Biomed. Pharm. Sci. , 2020, Volume 7, Issue 8, 126-127.*
- *William Winlow and Andrew S Johnson.* ***"The Action Potential Peak is Not Suitable for Computational Modelling and Coding in the Brain".*** EC Neurology 12.4 (2020): 46-48
- *Johnson AS, & Winlow W. (2019).* ***Are Neural Transactions in the Retina Performed by Phase Ternary Computation? Annals of Behavioural Neuroscience****, 2(1), 223-236. https://doi.org/10.18314/abne.v2i1.1893*
- 2019. **On the topic of mysteries of the action potential***;* Letters To Physiology Society News:Action potential conduction is not a mystery... **DOI: 10.36866/pn.113.6**
- *William Winlow and Andrew S Johnson.* **"Implications of the Action Potential Pulse Concept in Understanding the Mode of Action of Anesthetics"**. EC Neurology 10.10 (2018): 887-889.
- *Johnson AS and Winlow W. (2018)* **Mysteries of the action potential - From 1952 to infinity and beyond**. Physiology News. 2018;111: 38-41.
- *Johnson AS and Winlow W* (2018) ***The Soliton and the Action Potential – Primary Elements Underlying Sentience.*** Front. Physiol. 9:779. Doi: 10.3389/fphys.2018.00779
- *William Winlow, Munir M Qazzaz and Johnson A S.* "**Bridging the Gap – The Ubiquity and Plasticity of Electrical Synapses**". EC Neurology 7.1 (2017): 07-12.

- *Andrew S Johnson and William Winlow*. "Computing Action Potentials by Phase Interference in Realistic Neural Networks". ECNeurology 5.3 (2017): 123-134
- *Andrew S Johnson and William Winlow.* "Shortcomings of Current Artificial Nodal Neural Network Models". EC Neurology 4.6(2017): 198-200
- *A S Johnson.* "The Coupled Cardiac Action Potential Pulse (CAPpulse) – Synchronised Oscillating Mechanical Pulse Cardiac Action Potential". EC Neurology 3.6 (2016): 520-530

11. ADDENDUM.

This section contains my published papers not included in the main story but nevertheless providing extra material. I have also had several abstracts published for example for my presentation to the Physiology Society Long Covid Conference.

• The role of hemoglobinopathy in COVID-19 pathology. Fatemi R, Johnson A and Winlow W. Eur. J. Biomed. Pharm. Sci. , 2020, Volume 7, Issue 8, 126-127.

• 2019. On the topic of mysteries of the action potential; Letters To Physiology Society News:Action potential conduction is not a mystery... DOI: 10.36866/pn.113.6

• William Winlow and Andrew S Johnson. "Implications of the Action Potential Pulse Concept in Understanding the Mode of Action of Anesthetics". EC Neurology 10.10 (2018): 887-889.

• Johnson AS and Winlow W. b (2018) Mysteries of the action potential - From 1952 to infinity and beyond. Physiology News. 2018;111: 38-41.

• William Winlow, Munir M Qazzaz and Johnson A S. "Bridging the Gap – The Ubiquity and Plasticity of Electrical Synapses". EC Neurology 7.1 (2017): 07-12.

• Andrew S Johnson and William Winlow. "Computing Action Potentials by Phase Interference in Realistic Neural Networks". ECNeurology 5.3 (2017): 123-134

• Andrew S Johnson and William Winlow. "Shortcomings of Current Artificial Nodal Neural Network Models". EC Neurology 4.6(2017): 198-200

• A S Johnson. "The Coupled Cardiac Action Potential Pulse (CAPpulse) – Synchronised Oscillating Mechanical Pulse Cardiac Action Potential". EC Neurology 3.6 (2016): 520-530

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EC NEUROLOGY
Mini Review

Bridging the Gap – The Ubiquity and Plasticity of Electrical Synapses

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Abstract

- There is a substantial emerging literature on the complexity and plasticity of the apparently simple electrical synapses [ES]. Here we draw attention to some of the most recent findings in this rapidly evolving field.
- ES are ubiquitous, found in all multicellular animals and structurally underlain by gap junctions. Gap junctions are topographically similar in vertebrates and invertebrates, but based on different mutually exclusive connexins and innexins respectively.
- It is now clear that ES may be modulated and exhibit plasticity in addition to promoting synergy between coupled neurons according to the strength of coupling. Strong electrical coupling promotes synchronous activity while weak coupling may desynchronise coupled neurons.
- Chemical synapses may modulate ES conductances and may regulate the degree of coupling between neurons. Because ES act as low pass filters, prolonged spike after-hyperpolarisations can allow them to act as inhibitory connections, but modifications of conductances can allow them to act as high pass filters and there is gathering evidence that their gain can be modulated and is activity dependent.
- ES may be modulated by anaesthetics at clinically relevant concentrations and volatile anaesthetics can reduce coupling between strongly electrically coupled neurons in a dose dependent manner. This may prove to be important during anaesthesia, given the ubiquity of ES in the mammalian brain.
- ES appear to be seasonally modulated in the brain of the mollusc *Lymnaea stagnalis*, but the underlying mechanisms remain to be elucidated.
- Although neurons have distinct structures they do not necessarily act as single functional units and groups of electrically coupled cells may act as functional syncitia which suggests that Cajal's neuron doctrine and Golgi's reticular theory are not mutually exclusive.

Keywords: *Ubiquity; Plasticity; Electrical Synapses*

Ubiquity of gap junctions and electrical synapses

Gap junctions connect the cytoplasm of neighbouring cells and are found in most tissues of most animals with the exceptions of skeletal muscle, blood cells and other mobile cells. There were prolonged arguments in the early part of the 20th century about the nature of synapses, were they chemical (based on Cajal's neuron doctrine; [1,2]) or electrical (based on Golgi's reticular theory, [3])? The argument seemed to have come out in favour of chemical synapses by the 1950s, but at this point Furshpan and Potter demonstrated the existence of electrical transmission at the giant motor synapses of the crayfish [4,5]. Later it was revealed that gap junctions, which are now known to be the morphological structures underlying electrical synaptic transmission [6,7], are ubiquitous in the brains of mammals, fish and

birds [8,9]: "they are "distributed throughout the entire brain" [10]. Furthermore, they are found throughout the animal kingdom, e.g. in *Caenorhabditis elegans* [11], and there are functional descriptions in many invertebrates including the mollusc *Lymnaea stagnalis* [12]. However, there are clear structural differences between mutually exclusive vertebrate connexins and the invertebrate innexins which make up the connexons and innexons respectively [13,14], but both have four transmembrane domains [15] and intracellular N- and C-termini. However, the topographies of both types of molecule are similar [13,15] and in both cases the proteins may form hemichannels that mutually align to form gap junctions, through which molecules may pass from the cytoplasm of one cell to another. The related pannexins are not relevant to the present discussion, but more detail on them can be found elsewhere [13-15].

Recently Oshima., *et al.* [11] have demonstrated that in *Caenorhabditis* the innexin-6 gap junction channels are made up of 16 subunits, probably a general feature of all innexin channels, whereas chordate connexin channels are made up of 12 subunits. The implication is that innexin channels have potentially greater conductances than those of connexins. Interestingly, "the physiological properties of gap junction channels appear to be determined by the connexin expressed, independent of tissue type" [16] and this is probably also true of innexins. Within the vertebrate brain gap junctions are found between glial cells [17], particularly astrocytes, and between neurons and glial cells both in culture [18] and *in vitro* [17], although there is controversy as to whether these connections are fully retained in the adult brain. However, gap junction expression appears to be a requirement prior to normal chemical synapse formation [20,21] and both chemical synapses and ES may remain in close proximity to one another in the adult brain [22].

Modulation and Plasticity of electrical synapses

One of the main features of non-rectifying electrical synapses (ES) is the lack delay normally associated with chemical synapses for which reason they may promote synergy between coupled neurons [23,24]. However, plasticity of ES is demonstrable and they can be dynamically modulated by a wide range of neuromodulators such as dopamine, noradrenaline, glutamate [25], nitric oxide [26] and various other neuroactive compounds. For example, activation of metabotropic glutamate receptors caused reduced electrical coupling between neurons of the rat thalamic reticular nucleus [27]. In addition, chemical synapses may modulate the conductance of ES and thus regulate the degree of electrical coupling between neurons [28] and this voltage dependency [7] is likely to be a facet of specific connexins or innexins.

Although ES are apparently simple structures the emergent properties of coupled neurons may be highly plastic dominated by modulation of the biophysical properties of the cells involved [29], particularly the input resistance and the coupling resistance of the connected cells. Furthermore, the connexin (20 types) and innexin (25 types) proteins vary from one cell to another and influence the exact biophysical and biochemical properties of the junctions [13]. Phosphorylation of individual connexins, and by implication innexins, may modify cell to cell communication and movement of small molecules between the cells [30].

Do electrical synapses synchronise neural activity? It was previously assumed that the main role of ES was to synchronise neurons or groups of neurons [24,31,32], but due to the fact that they tend to act as low pass filters a deep and prolonged after-hyperpolarisation can allow them to act as inhibitory connections [33] and to allow them to synchronise or desynchronise the spiking activity of mouse Golgi cells depending on the input properties of the presynaptic signal [34]. Similar results have been modelled by Hull., *et al*. [32] for unmyelinated tadpole brainstem neurons where synchronization of rhythmic firing can be maintained or shunting through gap junctions may cause propagation failure. In other neural simulations, modification of the coupling coefficient may cause neurones to oscillate out of phase when weakly coupled and in phase when strongly coupled [35]. Strong electrical coupling between pairs or small numbers of neurons has been observed in the somata of mouse primary afferents located in the mesencephalic trigeminal (Mes V) nucleus and originating in jaw-closing muscles [23]. Here signals are enhanced by sodium and potassium conductances which allow the gap junctions to act as high pass filters, allowing the higher frequencies associated with the rising phase of the action potential to cross the gap junctions between the cells. This promotes strongly synchronised spiking to occur between Mes V neurons due to voltage dependent amplification of conductances which improve the efficacy of coupling. This finding supports the gathering evidence that the gain of ES may be varied

[25] and there is also evidence that the gain is activity dependent in the mammalian brain [36]. We conjecture that similar mechanisms may exist in other strongly coupled neurons, such as those in *Lymnaea* [31,37,38].

Further roles for ES Other types of interactions may also occur at ES. For example, rectifying electrical synapses were first demonstrated in the crayfish in 1959 [4]. More recently it has been suggested that ES may act as coincidence detectors in simulated systems [35]. In addition, interactions between chemical synapses and nearby ES can regulate electrical coupling at goldfish Mauthner cells [28] where the ES have an inhibitory effect due to their anatomical arrangement close to the Mauthner cell initial segment, altering the relative charge across the membrane [6,39].

Modulation of ES coupling by anaesthetics Given the plasticity inherent in ES, it should come as no surprise that they are also modulated by general anaesthetics in both astrocytes [40] and neurons [41]. Volatile anaesthetics applied at clinically relevant concentrations can reduce coupling between strongly electrically coupled neurons in a dose dependent manner by modifying their input resistance and other passive properties of their membranes [38]. An earlier study suggested that ES are less sensitive to most anaesthetics than chemical synapses [42], but Juszczak and Swiergiel [43] are of the opinion that anaesthetic compounds may confound behavioural studies because they block gap junctions, often in the clinical concentration range, although there are issues over which types of anaesthetic are most effective. Nevertheless, it is apparent that "suppression of gap junction function could compound the mechanisms of anaesthetic action" [41]. This would also be true in myocardial cells where the volatile anaesthetic enflurane is known to decouple gap junctions, perhaps altering conduction velocity and contractility [44].

Seasonal modulation of coupling ES appear to be seasonally modulated in the brain of *Lymnaea* [38,45] in which biogenic amines show dramatic seasonal variability [46] and in which chemical synaptic connectivity is also variable on a seasonal basis [47,12]. Temperature changes also have an effect on ES in *Lymnaea* as rising temperatures can reduce coupling coefficient between strongly coupled neurons [26], but this would appear to be opposition to the increased coupling found between these same neurons in the summer months [38], raising questions as to the mechanisms by which coupling is increased during warm weather.

Conclusion

Over the last two decades there has been a huge outpouring of research into electrical synapses which has demonstrated that ES are plastic, modifiable and of course ubiquitous. The very ubiquity of gap junctions indicates their importance in cell to cell communication in almost all tissues throughout the animal kingdom. If a phase model of computation is correct [48] then ES will have the direct role in computation of changing the phase of the action potential as it passes points of interference which will change the pathway in the neural network. Although neurons have distinct structures they do not necessarily act as single functional units and groups of cells may act as functional syncitia, sometimes firing in synchrony [49]. For these reasons, the neuron doctrine and the reticular theory are being revised into a new more holistic theorem of the functions of the nervous system.

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EC NEUROLOGY
Editorial

Implications of the Action Potential Pulse Concept in Understanding the Mode of Action of Anesthetics

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In recent publications [1,2] we introduced the concept of the action potential pulse (APPulse) which unifies our knowledge of the action potential based on the Hodgkin and Huxley [HH] model of the action potential [3] and the soliton pulse which accompanies it [4]. In earlier studies it was assumed that there were high enough concentrations of sodium channels in axon membranes to allow current to pass from one to another and to allow the action potential to pass smoothly along the membrane, but later work has shown that this is not the case [5]. Soliton pulses are now believed to mechanically distort sodium channels ahead of the action potential, allowing depolarization of the membrane and the smooth passage of the action potential once threshold has been achieved. Thus the APPulse is a combined electromechanical event where the speed of the soliton is determined by the membrane components and morphology. Furthermore, the soliton is capable of perturbation by external factors such as temperature or pressure [6]. Blockage to the APPulse can occur by blockage of the Na ion channels (as has been proposed for local anesthetics) and/or from blockage of the soliton.

According to Andersen., *et al*. [7], nerve membranes are an approximately equal mixture of lipids and proteins and two major theories of anesthesia have arisen. Initially the Meyer-Overton lipid hypothesis [8] seemed to indicate that lipids were the principal anesthetic target because of the correlation between anesthetic potency and their lipid/water partition coefficients. More recently it has been suggested that the principal effects of anesthetics are on ligand-gated channels [9]. This may in part be true, and we acknowledge that anesthetics have direct effects on ligand-gated channels. However, it ignores their likely effects on the voltage-gated channels that drive the action potential, which leads us to ask, what are the effects of anesthetic agents on voltage-gated Na channels in axons?

Heimburg [6] points out that biological membranes melt from a solid to a liquid state at physiological temperatures (although this phase transition must vary from species to species) which makes it possible for solitons to travel along nerve axons. He also indicates that both general and local anesthetics lower melting temperatures of membranes, making excitation more difficult. Wang [10] examined the effects of anesthetics on compound action potentials and action potentials from a single neuron and concluded that "Anesthetics move the chain melting transition temperature of membranes far away from the physiological temperature, which requires a higher free energy to induce the phase transition, resulting in a higher stimulation voltage to reach the maximum amplitude of the action potential". Furthermore, lipid channels displaying similar voltage clamp characteristics to sodium channels have been demonstrated in pure lipid membranes [11]. They are blocked by anesthetics and may add to the confusion over anesthetic effects on ion channels. We take the view that membrane lipids have a major role to play in our understanding of anesthetic mechanisms and would support the view that solubilization of general anesthetics in the lipid bilayer may cause a redistribution of the lateral pressures [12] that would normally cause opening of sodium channels during the action potential [2]. Others have suggested that clinical concentrations of general anesthetics do not have indirect effects on membrane protein function [13] and then assume that the effects on ion channels are direct, but they have not considered direct effects on the soliton itself. Since volatile anesthetics are applied to a cell in the clinical concentration range, i.e. at low

doses, their effects will not be "all or none". Thus, at low anesthetic doses the diffusion coefficient of Na will change the refractory periods of the action potentials making computation less stable. At higher doses solitons will be blocked preventing the APPulse.

In scientific research there are often conflicts between groups of scientists supporting opposing theories, but quite often the opposing theories, unify and a composite hypothesis emerges later. For example, in the 1950s it had just had become widely accepted that synaptic transmission was by chemical rather than electrical means when electrical transmission was conclusively demonstrated in the crayfish by Furshpan and Potter [14,15]. It is now known that gap junctions are the morphological structures underlying electrical synaptic transmission [16,17] and that they are ubiquitous in all multicellular animals [18,19]. Furthermore they exhibit plasticity and can be modulated by chemical synapses [20]. Given that electrical synapses may synchronize, or desynchronize the activities of groups of neurons, it is clear that although neurons have distinct structures, they do not necessarily act as single functional units. This in effect compromises the neuron doctrine [21] to some extent and partially supports the reticular theory [22] so that we now on the cusp of a new holistic unifying hypothesis for the function of nervous systems. Could the same now be true for our understanding of the mode(s) of action of anesthetics on the lipid/protein membranes of axons? If so, a better understanding of its various mechanisms yields the possibility of safer and more efficient forms of anesthesia.

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THE ROLE OF HEMOGLOBINOPATHY IN COVID-19 PATHOLOGY

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ABSTRACT
The novel coronavirus (Covid-19) is spreading rapidly worldwide. According to the World Health Organization, about 5.300 million people have been infected up to now and more than 340000 were died from Covid-19 infection (WHO, 2020). Every day, more and more unknown aspects of this "mysterious virus" are presented to researchers and clinicians. Based on available studies, the main pathology of the disease is that the coronavirus enters the cell by binding to ACE2 receptors and leads to proliferation and destruction of respiratory tract cells and pneumonia (Ge et al., 2013). Findings about the pathology of the disease are low and mostly have been stated based on assumptions. At the beginning of the disease prevalence, it was believed that the virus only involved the respiratory tract, leading to severe pneumonia and hypoxia, and finally death. However, recent reports suggest that in addition to the lungs, other organs, such as the heart, kidneys, eyes, and digestive tract, are also affected (Yuen et al., 2020). These findings have led to a revision of the pathology of the disease, and researchers are investigating new dimensions effects of the virus.

Based on previous findings about SARS and other viruses that infect the respiratory systemchanging hemoglobin values may be a predictive factor of worsening clinical progression in patients with Covid-19 because of significantly reduced hemoglobin values in these patients as well other types of pneumonia (Lippi & Mattiuzzi., 2019). Data from Liu and Li (2020) suggest that the virus attacks 1-Beta chain of hemoglobin, dissociating it from iron to form porphyrin so that less and less hemoglobin will be available to carry oxygen and carbon dioxide. Although theproposals of Liu and Li have been seriously queried by Read (2020) the implications of their hypothesis should be considered in some detail. It seems to us that destruction of hemoglobin may be one of the key roles played by the virus, because of the impairment oxygen delivery to vital organs such as the brain, heart, kidneys and liver. Given that the death rate of elderly patients succumbing to the virus is very high and that anemia is relatively common among those over 60 years (Lanier et al, 2018), further diminution of oxygenationto the organs may well explain the increased mortality. As we know, in many deaths caused by Covid-19 virus, tachycardiafirst occurred, followed by a fatal heart attack. It seems that after binding virus withhemoglobin, releasing ions lead to release free radicals which increase oxidative activity in the organs, leading to further hypoxia (Faiqet al., 2020). Many patients, who recovered and were discharged, still complained of shortness of breath and fatigue although their lungs were completely cleared of the virus (Ahmad et al., 2020).

Normally, hemoglobin delivers oxygen from the lungs and delivers it into tissues and organs. Each hemoglobin molecule contains four heme molecules, each of which binds specifically to oxygen in the lungs, during chemical interactions. The ions of iron (Fe2+or Fe3+) which are part of the structure of oxyhemoglobinare toxic in the free state and will increase blood oxidative stress (Emerit et al, 2001, Lippi et al., 2019). Assuming that the corona virus attaches to hemoglobin, FE++ or FE+++may be released into the blood and tissues, and this is where the main effects of the virus begin. In this case, the function of hemoglobin is impaired and the oxygenation process is diminished and corporeal hypoxia will constantly increase (Buoro & Lippi., 2018), perhaps explaining the shortness of breath and fatigue following recovery in some patients (Zhou et al., 2020). Decreased blood hemoglobin with elevated serum ferritin, erythrocyte sedimentation rate, C-reactive protein, albumin, and lactate dehydrogenase also low oxygen saturation may be evidence of this hypothesis. Patients'

low oxygen saturation even when a ventilator is employed is further evidence (Faiqet al., 2020, Lippi & Mattiuzzi., 2019, Chen et al., 2020). The other reason for this assumption is that based on the study by Lansiauxet al., 2020, which indicates that beta-thalassemia subjects are immunized against Covid-19. Beta-thalassemia results of a default in the hemoglobin beta- chain synthesis, indicating that Covid-19 attacks beta chain of hemoglobin.

It may be that the main reason for the appearance of patchy consolidation and ground glass opacities in the lungs (Kong and Agarwal, 2020, in press) is due to tissue damage caused by the presence of these free ions and consequently free radicals. The epithelial surface of alveoli containshigh volumes of antioxidant molecules such as nuclear factor-erythroid 2 related factor 2 (Nrf2) (Kosmider et al., 2012), but these may not be sufficient to counteract those derived from damaged hemoglobin.

In patients suffering from Covid-19, we would recommend that oxidative stress indicators and the amount of antioxidant enzymes and oxidative products in these patients be evaluated.

Blood transfusions or sera transfusions may need to be performed regularly in such patients while maintaining them with hyperbaric oxygen.

Author contributions

RF, ASJ and WW conceived and designed, read and approved the submitted version of the study, RF wrote the preliminary manuscript, WW and ASJ contributed to its technical revision.

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Conflicts of interest

The authors declare no conflicts of interest.

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EC NEUROLOGY
Opinion

Shortcomings of Current Artificial Nodal Neural Network Models

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The usefulness of small-networks to model large-networks is limited in biological systems and synaptic studies give little insight into conduction in more highly evolved brain-neural-networks where axon conduction is diverse and seemingly unreliable [1-3] with an alarming amount of noise, reduction of which must be taken into consideration for any neural network of depth. Reverse engineering models [4,5] assume processing works like a conventional binary computer and neglects speed of cognition, latencies and error in nerve conduction and the true dynamic structure of the brain neural network: any model of nerve conduction that claims inspiration from nature must include these prerequisite parameters.

Historically research has followed the progression of computational science; literature film academic and non-academic articles almost universally speak of the action potential being binary and binary mathematics has been assumed by force of popular acceptance to mediate computation in the brain. A superficial examination of the history of computing and the action potential itself quickly dispels these assumptions; at the time the action potential was discovered the field of computation was pre-transistors and any base or indeed analogue was considered though not a compound ternary-phase pulse structure. The availability of cheap binary transistors ended investigation into ternary computing. Because of the latencies involved in slow moving pulses, phase computation was not considered practical [6]. However this predated understanding of relational-databases or neural networks and their function. For the brain neural network to function as an associative database (multiple associations) it must have a depth of many thousands of nodes allowing multiple associations within the first layer mounting exponentially with every layer. Using the compound phase action potential within a deep small-world neural network overcomes the restrictions of synapse mediated memory redacting error, and counter-intuitively as depth increases timing between association decreases, so that the deeper the network the more efficient it becomes in respect of time. This is a natural consequence of the small world network where collision points represent the nodes so that every possible conceivable connection from a node can be represented as a potential associated memory. Computation in a network may occur in a number of ways and a binary notation is not exclusive, logic may use other base forms and timings. Superficial calculations on timing, facility of computation and error demonstrate that only a few current models are applicable to vertebrate brains or those of advanced invertebrates and almost all can be immediately discounted.

Reasons for reinterpreting and modifying artificial nodal network models.

1. The modus operandi of action potentials is unlikely to be binary and is probably driven by compound ternary structures.

2. Depth of neural network– the human neural network is unrestricted in its ability to learn sequentially. Histology and genetic studies demonstrate that the neural network is not fixed, is randomly connected and randomly assigned directionally. In addition plasticity ensures that no one connection can be considered 'fixed'.

3. Fixed latencies between neurones– the neurons of the brain are of different sizes, length and composition; it is almost impossible for action potentials to arrive synchronously. The speed of axon transmission is discussed in more detail in the Action Potential Pulse [7].

4. Speed of cognition and speed of connections [8] – the modelled computational speed of an artificial neural network cannot explain the speed of cognition and learning unless speed of connections is ignored.

5. Energy requirements– artificial neural network modelling of synapses to produce 'weighting' is inefficient, as it requires additional steps for computation and timing.

6. Algorithms or processes for each decision indicate that a further mechanism must be instigated. Bayesian calculations are time-inefficient, at action potential speed, to produce a weighted result and there is little evidence such a system works in the brain– there is also no evidence of any corrective mathematics in the brain that would compute as software or redact error.

7. Error– use of synapses creates additive error and inefficiencies in the network making a very deep unsupervised learning network unreliable. Error in the neural network approaches that of activity but memory in both animals and humans persists over many years with a high degree of accuracy indicating that error is redacted. Using synapses as gates creates analogue error that would prevent an associative matrix and further decrease the level of memory and sequential computation. Balanced ternary phase computation in contrast natively reduces error to zero by parallel pathway negation of "of-of" synchronisation of action potentials.

8. Genetic considerations. There are not enough genes to denote positions of any but main pathways – neurons and connections are likely to be positioned randomly and multidirectionally.

9. Plasticity in the system where synapses hold memory would act detrimentally. In a phase system plasticity enhances system efficiency providing additional pathways and memory where and when required.

10. Measurement of memory needed to sustain cognition in a network. The number of synapses would be barely enough to sustain a life using synapses to control or retain memory when considering that there must be a large proportion given over to calculation and error – none of which is covered adequately by conventional models.

In the near future we will examine these networks and the above assertions in further detail and make suggestions for their modification. The nature of action potentials has already been discussed in the context of neural network efficiency [7,9].

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Research Article

The Coupled Cardiac Action Potential Pulse (CAPpulse) – Synchronised Oscillating Mechanical Pulse Cardiac Action Potential

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Abstract

Fundamental to the functioning of the heart its efficiency and lifetime durability is the control of its speed of contraction. Contemporary thinking supposes that contraction occurs by the Cardiac Action Potential (CAP) that can be measured easily and has its origins from trans-membrane 'ion-currents' propagating through ion channels along the surface of a membrane. However, as this study demonstrates mathematically and empirically, cable theory is an imperfect model for describing the AP and for subsequent electromechanical coupling in the heart. Assumptions of almost instantaneous activation of progressive ion channels to produce the Cardiac Action Potential are wrongly based upon electrostatic charge travelling from one channel to the next at or near the speed of transmission: empirical evidence from channel spacing, ionic radii and diffusion coefficients demonstrate mathematically that this is not the case. Further scrutiny is therefore required, particularly as one in four deaths is caused by cardiac irregularity. However, evidence exists from ion channel studies and entropy measurements of membranes for a synchronous oscillating cardiac action potential mechanical pulse structure that is efficient and fully explains the speed of transmission: this paper proposes this model as a foundation for consideration in further studies of the heart.

Understanding of the activation spread and speed of contraction is essential in any study or clinical application of the heart. The Coupled Cardiac Action Potential Pulse explains these mechanisms. This is a more consistent model for cardiac pulse activation: it is consistent with observed studies and the principle may be applied to other types of muscle.

This paper is a derivative of: The Action Potential Pulse (APPulse) – Synchronised Oscillating Mechanical Pulse Action Potential [1].

Keywords: *Synchronised Coupled Oscillating Pulse; Cardiac Action Potential; Membrane; Mechanical Pulse CAP*

Abbreviations

AP: Action Potential; CAPPulse: a coupled oscillating mechanical pulse formed from the activation of muscle; HH AP: Hodgkin-Huxley Action Potential. Speed of pulse is defined by a lipid pulse and initial activation of the ion channels to instigate potential threshold leading to full depolarization is by predominantly mechanical forces, with an electrical component providing activation in membrane-areas of very high channel density - which almost certainly includes an entropy component created by cardiac muscle contraction.

Introduction

One in four deaths is caused by cardiac irregularity; ventricular arrhythmias are a major cause of sudden death, which accounts for approximately half of cardiac mortality; speed of contraction and force developed are general quantifiers of cardiac health.

The Cardiac Action Potential conduction system was adapted from the elegant theoretical model of Hodgkin and Huxley [2] that forms the basis of the textbook accepted mechanism for the action potential (AP) along the membrane of nervous tissue. Evolution of their model has progressed according to the availability of experimental data with the assumption that their work was essentially correct, although

it was accepted that the speed of transmission could not be defined. Their paper assumed cable theory in the action of propagation to provide the speed of impulse along the axon although little or no biological evidence exists to corroborate this.

Similar membrane constituents make up the membrane structures that power the Cardiac Action Potential (CAP). The mechanisms that power both the AP and CAP can be thought to be analogous but are by no means similar because cardiac muscle is fundamentally a moving structure that during the CAP-contraction changes size and shape - although similarities exist in the membrane channels and fundamental materials of the membranes. Previous work has shown that a 'soliton [3,4]' lipid mechanical pulse [5-8] may be formed from the entropy fluid-mechanics of the lipid membrane, and moves at a rate almost indistinguishable from that of the HH AP in axons and the membranes of sarcolemma are similar.

Entropy measurements and the large spacing between ion channels discredits cable theory leaving the only plausible explanation for speed of propagation to be a combined and synchronised oscillating pulse [1], making a closer critical look at the CAP a necessity.

The basis of this paper is to examine the contemporary mechanism proposed for the Cardiac Action Potential CAP specifically the CAP actinomyosin coupled contraction in relation to the speed of systole with the hindsight of knowing that ion channel propagation alone cannot explain speed of the CAP. Timing between systole and diastole and indeed pulse rate are critical indicators of heart health and the fundamental mechanism essential groundwork before any understanding of biochemical or pharmacological investigation.

The discovery of the APPulse [1] led to the reinvestigation of the inter-channel distance between ion channels in the heart throughout the cardiac syncytium which are of a similar magnitude. Similar mechanisms may be shown to mathematically exist to the APPulse where continuous depolarisation along the axon membrane is impossible without a further mechanical element. This forms the elements of the Coupled Cardiac Action Potential Pulse (CAPPulse). Speed of contraction and synchronous timing is fundamental to the proper and efficient functioning of the synchronicity of cardiac muscle. Any change in this synchronisation leading to inefficiency may lead to enlargement, deterioration or even failure. To understand the controlling mechanisms that define the speed and synchronicity of function it is necessary to deconstruct the flow of the Cardiac Action Potential which forms the basis of conduction and to evaluate those elements necessary for initiation and those that mediate effectiveness. For this to happen an accurate model must be formed that can then be used to evaluate the processes that affect the mechanical operation, pharmaceutical modifiers and applied experimental techniques.

The cardiac impulse (CI) is generated in the sinoatrial (SA) node. It traverses and activates the atria, before converging on the atrioventricular (AV) node for distribution to the ventricles via a specialised conduction system; the bundle of His; the main bundle branches to each ventricle, and the Purkinje fibre network. Originating in pacemaker cells the CI travels along the His –Purkinje system composed of specialised cells responsible for the synchronous activation of the ventricles and timing of systole [9,10].

Contemporary orthodoxy separates the cardiac action potential and the His –Purkinje system from the contraction of the underlying muscle although it has been noted that the speed of the action potential, in normal function, is tied precisely to that of the contraction and that contraction once instigated may proceed when the His-Purkinje system is incapacitated [11]. Experimental studies show that the Purkinje system can be arrhythmogenic during electrolyte imbalance, after exposure to various drugs, and in myocardial ischemia, during which Purkinje cells can survive in anaerobic conditions [12,13]. In addition, catheter ablation where the conduction system of the Purkinje tissue is severed can often prevent cardiac arrhythmias.

The Cardiac Action Potential differs significantly in different regions of the heart, reflecting a differentiation of functionality that provides both synchronised and concatenated contraction of disparate areas [14]. There are many reviews of the mechanisms involved of Pacemaker cells, Purkinje cells and the corresponding pathways of activation that are outside the scope of this paper.

An understanding of the physiology of ion handling in cardiac cells is critical for understanding the functioning of the excitation-contraction (EC) coupling of cardiac muscle. Central to this is the conventionally accepted mechanism of Ca induced Ca release (CICR).

During a CAP the voltage dependent L type Ca channel is activated – resulting in a small influx of external Ca into the cytosol. This Ca bids to the cytosolic Ca ryanodine receptor RyRs located in the sarcoplasmic reticulum. Ryanodine receptors (RyRs) exist as three mammalian isoforms (RyR 1–3) of which RYR2 is predominant in cardiac muscle. RyRs are located in the sarcoplasmic/endoplasmic reticulum membrane and are responsible for the release of Ca^{2+} from intracellular stores during excitation-contraction coupling in both cardiac and skeletal muscle. RyRs opens channels leading to an out-flux of Ca from the SR – the main intracellular store of Ca. The released Ca then binds to Troponin C causing a cascade of conformational changes in the myofilaments and ultimately causes muscle contraction. During the relaxation phase Ca release is terminated and the released Ca is recycled back to the SR by the SR CA ATPase or extruded by the cell by the Na/Ca exchanger thus lowering cytosolic Ca concentration and allowing dissociation of Ca from the myofilaments permitting muscle relaxation [9].

Recent studies [11,15] have shown that many membrane channels are also mechanically gated or in the case of more than one stimulus mechanically modulated [11]. Lipid Channels may also form under mechanical stimulation [16].

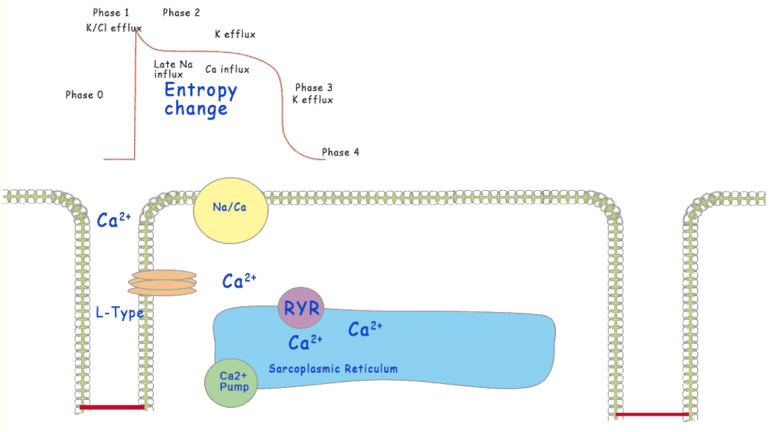


Figure 1: *Resting State.*

In the conventional model of Excitation Contraction coupling, excitation of the adjacent membrane causes potential sensitive channels to open unrelated to the likely physiological impact of contraction from myofilaments situated within the local or adjacent syncytia. Neither is the CAP considered to be anything greater than a potential difference acting upon potential-sensitive channels: neglecting the impact and possibility of a lipid pulse travelling along the membrane or of the undeniable effect of the myofilament contraction upon the spatial histology of the syncytium, sarcoplasm, lumen and the ensuing mechanical effect this would have upon the internal structure and function of the SR, T tubules and the cellular Ca constitution.

This paper examines the relationship between the ion channels and the membrane spread of the CAP critically in relation to the timing of the opening of the channels to enable full depolarisation. In the CAP the speed of spread across the membrane is dependent upon a rate limiting 'threshold'. This is the absolute moment before hyperpolarisation is irreversible and is implied to be the electrostatic opening of Na gates – phase 0. Rapid Na^+ channels are stimulated to open, flooding the cell with positive sodium ions. For this hyperpolarisation to occur there must be a mechanism to open successive channels across the surface of the membrane. Cable theory suggests resistance to charge flow as being cross membrane – as in an electrical circuit – but it must also include flow of ion charge from one channel to the next for hyperpolarisation to occur. By contrast to an electrical circuit where spread of electrostatic charge across a capacitor membrane is almost instantaneous positive ions have to physically move – capacitance therefore is a useful but inaccurate model. No electrons are

involved in the process and the difference in potential is achieved by relative potentials of positive ions. Positive ions are of a defined size and require a finite time to travel not only through the membrane but also to adjacent channels in order to activate and eventually hyperpolarize the membrane. It is the mathematical relationship between the distance and the time taken that defines the spread across the membrane and which can be shown to be reliant upon further factors. This relationship is easy to calculate from ionic radii and diffusion coefficients both of which are available.

This paper firstly describes the general, mathematical relationship between the CAP and contraction in terms of membrane potential and examines the possibility of CAP spread and hyperpolarisation along the sarcolemma by a coupled mechanical synchronized pulse. This coupled synchronised oscillating potential-mechanical membrane compression pulse is comprised of; the potential difference across the membrane, a lipid pulse allowing the potential to spread and hyperpolarize and 'electro'-contraction-coupling caused by contraction of the myocytes and the deformation of the syncytial membrane leading to a synchronised CAP Pulse where contraction: a. deforms the membrane causing mechanical opening of the channels and a CAP, b. compression of the sarcoplasm and sarcoplasmic reticulum within causing expulsion of Ca leading to myocyte contraction.

Implications of this model include an explanation of Delayed after depolarization (DAD) caused by enforced delay between the CAP and the on-going contraction pulse causing a state of duplication of the CAP and defibrillation.

Methods

An examination was undertaken into the historical significance of research into the cardiac action potential, its flow across a membrane and its relevant inclusion into a scheme for cardiac action potential flow with respect to recent discoveries of ion channel activity.

The maximum speed of hyperpolarisation of the membrane was calculated using the method-values published from single Na and Ca ion channel studies ionic radii and diffusion coefficients.

The speed of activation of the myocyte was calculated from the point of hyperpolarisation by a calculation of the diffusion coefficients, ionic radii and the approximate length of T tubules thought to provide intracellular Ca^{2+} to act on tropomyosin complexes and induce myocyte contraction.

A model of membrane activation and myocyte contraction was formed that was consistent with the experimental studies and the calculated results.

Where inconsistencies became apparent, mathematical models were created consistent with measured entropy, ion channel activation, lipid pulses and efficiency.

Calculation was undertaken on ionic diffusion coefficients to show the time required for ions to travel from one channel to the next to activate hyperpolarisation. Diffusion mathematics is well defined and documented and beyond the scope of this paper, calculations are given in results. Ionic radii of ions were also evaluated to verify the extent to which one charge can affect another at distance. Methods of single patch clamp studies were researched to determine the average distances between ion channels [17,18]. Calculations were then made to determine whether charge from one channel alone could open a neighbouring channel and thus be responsible for the CAP and cardiac contraction [16,19,20].

Results

For continuous excitation and hyperpolarisation charge must reach progressive ion gates to open them. Unlike electrical circuits where electrons spread across the metallic sheet of a capacitor at near light speed positive ions have a limited ionic radius and must either be adjacent to, or physically move into position to affect the next channel. This follows for all electrostatically operated channels.

In cable theory terms the threshold is the potential caused by the capacitance potential of the main digit caused by ionic charge and creating all-or-none equilibrium activation. The capacitor aspect of the Cardiac action Potential is accepted as creating the digit potential; of

contention is how the charge flow from the initial digit can cause the continuous contiguous depolarisation along a membrane from one ion channel to the next in the time measured by the speed of the propagating cardiac action potential. Ions require time to flow by diffusion through and along the membrane surface before activation and further propagation of consecutive ion channels are opened creating exponential hyperpolarisation phase 0 of the CAP Na intake. It is this process that defines the speed of the CAP linked to the speed of cardiac contraction before repolarisation and recovery. The ability of ionic charge flowing through one ion gate to affect another is dependent upon how fast that charge may spread. Each ion channel and direct pathways between channels represents an individual resistance to this process so that total charging time T of the capacitance representing the exponential rising phase of the digit:

$T = \sum t.$

Where t is the mean time taken for adequate charge to spread from one channel to the next and activate exponential threshold.

Patch clamp studies demonstrate that single ionic activation channels are typically greater than 1 μm apart [13,21] with 1.5 μm taken as standard for pipettes in single ion channel clamping research [13]. Usage of pipettes with a tip diameter greater than 2 μm results in unpredictable results when more than one channel becomes covered by the pipette. This empirical measurement has therefore long been established and accepted as being the distance between adjacent channels. Time taken for the propagation of the ionic driven action potential along the membrane is dependent upon the speed that ionic charge may be transferred from one ion channel to another by diffusion or by the ionic radius of the ion so that in membrane ion channel threshold terms mean speed is defined by:

$$S \propto \frac{\sum I+D}{t}$$

where S is the speed, I is the ionic radius and D is the diffusion distance.

For release of ions from one ion channel protein to affect another, cause hyperpolarisation and charging of the membrane requires time t. The time taken for ionic charge to spread from one point to another can be calculated from the rate of diffusion and the ionic radius of the ion.

The time for diffusion of an ion can be calculated approximately using the formula:

$T \approx x^2/D$

D is the measured diffusion coefficient of the ion, T is the time taken and x is the mean distance. The ionic diffusion coefficient of Na^+ is 1.33×10^{-5} cm^2/s [19,20] and that for Ca^2+ 0.79 × 10-5 cm^2/s.

For the sodium channels: Substituting mean distance between ion channels of 1 μm gives an approximate diffusion time of 0.3ms, which marks the maximum speed of transfer of the first ion charge out of the proximal ion channel to the first ion to the distal channel.

The ionic charge surrounding charged atoms that additively combine to give the digit potential hyperpolarisation has an effective distance time spread that is fixed by the ionic radii in which to stimulate other molecules (ion gates). The ionic radius (the distance over which each charge may be measured effectively) is only 116 picometres(pm)or Na^+ [21,22] and 114 pm for Ca^{2+}

Thus, the speed from diffusion is insufficient to affect distal ion channel activation timing and continuous flow cannot be achieved by a CAP model alone as a CAP it would require a diffusion coefficient of about 5000 cm^2/s assuming a distance of 1.5 μm. Using $T \approx x2/D$ and substituting Ca diffusion coefficient of 0.79×10^{-5} then it would take a Ca ion 7325 days to travel a metre [11,15] or more simply take about 1 year for each heartbeat.

CAP Channel Proximate Action Hyperpolarisation

Instigation of depolarisation is by pacemaker cells. For a CAP to exist as an entity and to progress along a sarcolemma depolarising and causing hyperpolarisation the channels would have to be much closer together. It is possible to accept that channels may open as a result

of ions proximate to the sites channel receptors – within ionic radii or, even allowing for charitable error double ionic radii. However, it is not possible at initial activation threshold for ionic charges to spread across the membrane in sufficient quantity and time to activate ion channel receptors in the time taken for moving depolarisation as demonstrated by phase 0 of the CAP – the distances in consideration of the diffused-charge distance are too great.

The conclusion is that ionic charge alone cannot dictate the forward moving depolarisation speed but only defines the shape from threshold to hyperpolarisation, in other words the large entropy, E.

It is therefore highly unlikely that charge from the digit leading edge of the CAP is the responsible instigator for activating threshold hyperpolarisation during CAP active flow. Another process therefore must bring the action potential to threshold during AP flow and regulate speed of CAPPulse, and myocyte contraction and be responsible for the mechanical time/force physiological qualities of the heart. The CAP alone is not therefore responsible alone for the activation of either the sarcoplasmic reticulum or of myocyte contraction.

The Coupled Cardiac Action Potential Synchronised Oscillating Mechanical Pulse (CAPpulse)

Cardiac muscle is a mechanical entity, its function is to contract, exerting pressure: as a direct consequence of contraction physical changes take place within the cellular structure. Cells in the human body are not fixed but are composed of membranes, protein and compounds almost all of which exhibit elasticity and subsequently hysteresis in relaxation. In the APPulse [1] it was described how a coupled lipid action potential pulse could better describe a model for an action potential than that of Hodgkin Huxley and could furthermore better explain myelinated transmission. Similarly, the sarcolemma of a cardiac syncytium is a lipid membrane where a lipid pulse could explain CAP hyperpolarisation. However cardiac muscle itself is a moving component pressurizing the syncytium many times during contraction which undoubtedly has an effect both on the CAP travelling along the membrane and the spread of Ca through the sarcolemma to the sarcoplasmic reticulum and almost certainly in Ca activated Ca release.

A model is therefore proposed combining these three factors, CAP, lipid pulse and mechanical contractive cellular changes. A preliminary discussion of the soliton pulse is described elsewhere [1,3,16,25]. Many occurrences of synchronous oscillation appear in nature and a mathematical treatment of them has been reported elsewhere [26,27,28].

A coupled cardiac action potential pulse is formed when a threshold is reached by mechanical stimulus with a HH digit that provides the energy required for on-going entropy of the pulse. Entropy: The total entropy E of the system where an initial burst of entropy is dissipated e resulting in enough entropy to open the ion gates restarting the process.

For the benefit of a clear description the sarcolemma has been standardised as follows:

a. The sarcolemma is uniform such that speed along the sarcolemma by the lipid pulse is constant.

b. The protein channels are gates that reach threshold at a voltage of V and produce a digit of Entropy E distance along the sarcolemma is proportionate to time.

c. In this sarcolemma, there are no lipid channels or other proteins except the three ion channel proteins.

Figure 2 demonstrates that on depolarisation at a, an action potential digit of entropy E is created. A Lipid pulse wave is subsequently created by Entropy E that continues along the sarcolemma. Entropy loss e from E causes a proportionate decrease in amplitude but not in speed according to wave theory [17] that causes the entropy to decrease by dissipation over distance d such that Ed(b) = E-e. This residual entropy Ed(b) is above threshold t and causes depolarisation at b to complete the circle. In this model the entropy of the CAP digit must be sufficient to produce a lipid pulse of such entropy E to arrive at b and take b above threshold for the CAPPulse combination to continue. Continuance of the pulse depends upon the entropy provided by the HH cycle – the digit and the entropy loss e of the lipid pulse.

If the entropy provided by the ion protein channels is sufficient only to provide a pulse of entropy E that produces less entropy than that required by t then continuance of the pulse will fail.

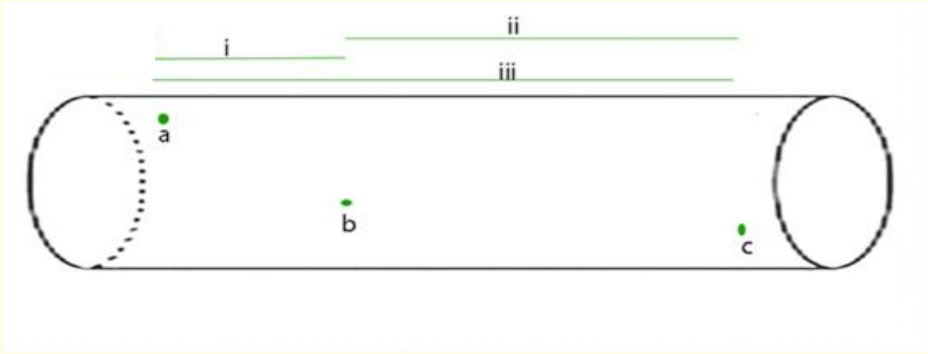


Figure 2: *An illustrative, uniform sarcolemma containing three widely spaced ion protein channels.*

A random placement of ion channel proteins produces a mean oscillating synchronised CAPPulse from the HH depolarisation and the lipid soliton. This Oscillation between randomly placed ion channels produces a structure where the dynamic at any finite point on the sarcolemma produces a differential CAPPulse that has an absolute value (the threshold t) a variable entropy (an initial burst E followed by a level of decreasing entropy e) depending upon the specific dynamics of the membrane. At that moment, the concentration and distribution of ion protein channels and the lipid surface of the membrane will considerably change the transmission properties of the pulse and at that location the dynamics may allow either an HH AP a Lipid Pulse or an oscillating synchronicity.

Timing

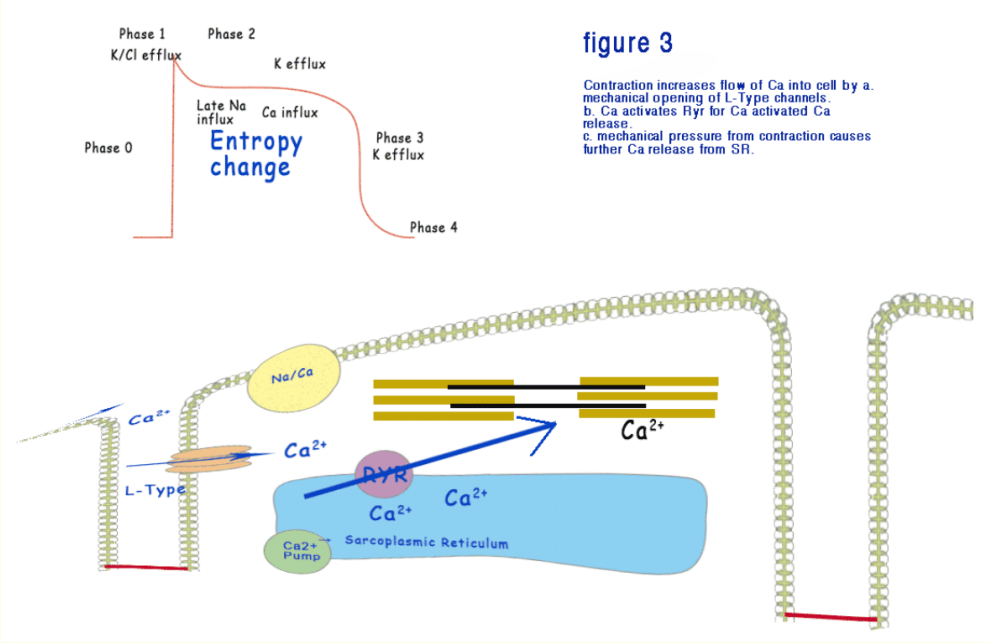
In a Coupled Oscillating Cardiac Action Potential Pulse, it is the lipid pulse phase that provides the overall speed of the pulse. The time t entropy E and decay e values of this pulse are therefore different for each sarcolemma (part) giving a distinct profile. This lipid pulse is also affected by the cardiac muscle mechanical contraction and is almost certainly bound to it unless the membrane is ruptured or malformed producing further coupling. The shape profile of this pulse is almost infinitely variable depending upon the dynamics of the sarcolemma explaining differenced in recorded CAP: both the lipid structure, the HH components and the other elements forming the membrane. The t E and e curve therefore is almost certainly non-linear but theoretically stable for any defined stable point on the sarcolemma. Computationally this can be reduced to a variable threshold value for HH and the continuance of the CAPPulse at any distance along the sarcolemma.

Timing is therefore oscillatory between the three structures at a set point and coupled by entropy provided by the HH CAP and the mechanical component.

Ca activated Ca release by CAPPulse:

The exact sensor for intra-SR release of calcium has not been elicited but is suspected to be the ryanodine receptor described briefly above it is also known that many channel proteins exhibit mechanical opening under pressure [11,15] including the L type Ca channel and ryanodine [11]. Histologically the SR is a connection of tubules within a finite space with the SR placed in close proximity to the T-tubules allowing for close connectivity between a CAP pulse and the SR and rapid pulse transfer. Partial de-tabulation of the muscle causes a depressed contractile force and slower twitch kinetics [29].

There are two main ways to change the strength of cardiac contraction: by altering the amplitude or duration of the Ca transient, and by altering the sensitivity of the myofilaments to Ca. Myofilament Ca sensitivity is enhanced dynamically by stretching the myofilaments (as the heart fills with blood), resulting in a stronger contraction. This is due, in part, to the transverse filament lattice compression that occurs on stretch, which enhances the actin–myosin interaction. This lateral compression is an important self-regulatory mechanism by which the heart modifies to alter diastolic filling.



A sarcolemma is made up of many ion protein channels that may have more than one activation method; in addition there are lipid channels [30], and a mechanical wave pulse accompanies the action potential [23,25]. Membranes possess many electromechanical properties that must change the basic flow of ionic charge. It is probable that in the flowing CAP the remaining entropy e of the lipid pulse soliton activates the channels to threshold potential by mechanical means as well as the ionic charge flowing through the channels to hyperpolarisation. This would be analogous to the hysteresis mechanical effects on substances like a rubber band that once deformed takes time to restore to its original shape, thus delaying closing the measured refractory period.

After activation of any part of the syncytium by the sarcolemma, internal pressure in any part of the SR will rapidly transfer causing a spread of Ca greater than by diffusion alone. It is this pressure that opens Ca channels and precipitates Ca activated Ca release; in this model, it is unnecessary for large potential changes within the syncytium for SR-Ca activated Ca release. The combination of potential change and pressure opens the SR channels. In this model, any proximate change in charge in any region of the SR instigates an immediate pressure curve to open other Ca channels. The rate of activation is proportional to the pressure and the levels of Ca. Closing may be timed by hysteresis.

This model is consistent with the Frank Starling effect [31] where the stroke volume increases with the filling of the heart due to the extra pressure within the syncytia promoting an increase in internal fluidity.

Conclusion

It is an empirical impossibility that the CAP is the correct activator of cardiac muscle; whilst the CAP is alluringly simplistic it is unacceptable as a foundation for research into an organ responsible for the death of a quarter of all human life.

Without an electrostatic charge, sufficient to open ion channels, the only consistent mechanism for the activation to threshold during depolarisation is mechanical displacement caused by the entropy from a lipid soliton in coupled action with mechanical contraction. The Hodgkin Huxley equation explains the initial entropy but cannot account for the continuous depolarisation speed of propagation or contraction, as it does not take into consideration the spatial membrane dynamics of the ion channels or explain activation.

The Coupled Cardiac Action Potential Pulse is an oscillating pulse powered by the entropy of the HH cycle that flows through a lipid membrane between channels. This pulse flows down the T-tubules opening the Ca channels that in turn activate the channels in the sarcoplasmic reticulum. In this respect, there is little evidence of excitation – contraction coupling but of coupled excitation-pulse contraction coupling. Spread of Ca from the SR is by initial diffusion and then by pressure pulse fluid movement within the lumen with mechanical forces opening channels. Ca is recycled by the Na/Ca and Ca ATPase within the membranes.

Although the CAP permits clinicians to view certain aspects of cardiac activity, what is seen is a ghosting of actual cardiac function. Ironically in a healthy heart the CAP records correctly and mirrors the CAPPulse, however during cardiac arrhythmia or DAD for example, what is recorded is the CAP and not the activity of full cardiac function of the pulse and flow of activation or activity within the sarcoplasm.

Synchronicity is of importance to the heart to maintain cellular integrity; like all materials undergoing stress movement within the membranes and proteins of the heart must be kept within normal limits to prevent stress injury. Cardiac muscle in desynchronised or syncopated rhythm, like any material, produces stress and repetitive strain. Membranes subjected to repetitive action are susceptible to injury.

Under normal working conditions the feedback oscillation from the coupled CAP – pulse – contraction complex maintains a synchronised heart, however it is not difficult to see that where the CAP is cut from the pulse contraction or the CAPPulse from the contraction any split will produce two contractions; these extra contractions then become the basis of arrhythmias and DAD. Any slight de-synchronisation will inevitably damage tissue further.

This coupled pulse has all the elements of the Hodgkin Huxley system except:

a. Less ionic protein channels are required.

b. The system is stable.

c. Less ionic current is required to operate the system.

d. Less energy is required as the entropy provided by the HH depolarisation is used to 'power' the APPulse pulse. In addition, cardiac contraction itself contributes to its entropy.

e. Unlike HH alone on-going soliton pulse activation of the ion channels predicts a cardiac action potential pulse of the correct speed.

f. It is more efficient as it only requires a pulse of entropy from an ion channel followed by dispersion by the lipid pulse – the lipid pulse also is coupled to the cardiac contraction.

The theoretical joining of the 'soliton lipid' mechanism, the HH AP and contraction adds considerably to the efficiency of a pulse and increases the accuracy and efficiency. Such a system would combine the efficiency and consistency of speed of a wave pulse moderating the speed of impulse with the control of an ion flux system extending the distance over which the soliton mechanical pulse would flow by addition of entropy. The dualism of soliton-HH would greatly enhance efficiency and reduce variability of impulse latency in finite time even without contraction.

Clinical Implications

Clinicians use their knowledge of cardiac physiology to predict the actions of pharmaceuticals on heart rate/force: this model changes the application of treatments to better reflect the actions of the cardiac muscle allowing more accurate treatments to become available.

Knowledge of the CAPPulse should provide information for a new type of synchronised pacemaker to control arrhythmias.

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Volume 3 Issue 6 November 2016