On Significant Discoveries and Landmark Experiments in the Development of Modern Neuroscience

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Abstract- The methodologies utilized in the contemporary study of neuroscience are manifest from the collective efforts of centuries of determined and insightful researchers. From the first query into the most basic of all mysteries, *what is a soul,* scientists have struggled to narrow the search in what is an ever expansive library of possibilities. The early works of Galen, Descartes, and Newton helped focus that attention toward the brain and the elaborate network of interconnections the brain uses to interface with the outside world. Later works of Reymond, Hermann, Helmholtz, Hodgkin and Huxley, and many others, helped form the framework of the current understanding of neuroscience. This paper will attempt to explore the contributions of the critical physiological experiments that facilitated the advancement of this understanding. It is the further goal of this paper to establish, by citation of the discoveries throughout the history of neuroscience, that the contemporary understanding of the activation, creation, and conduction of action potentials through neural tissue is valid.

***Index Terms*— History, neuroscience, neurophysiology, electrophysiology, membrane potential, action potential.**

I. Introduction

IN 159 C.E. a young physician was appointed the personal doctor to Marcus Aurelius, emperor of Rome. He was to care not only for the Emperor but for the gladiators who fought for his amusement. Over many years he made tremendous contributions to the understanding of human physiology by working mostly with the Barbary ape, and applying those discoveries to humans [1]. He would discover that the kidneys produced urine by tying the ureter closed and observing the kidney swell. He would go on to discover that paralysis of anatomical structures would result after severing the nerves that controlled them, though he did not fully understand their functionality. These discoveries seem so trivial in the 21st century but to Galen, the young doctor, they were revolutionary. Still many others have contributed in this manner and the contemporary model of biological processes is indebted to their discoveries.

As with all scientific progress, the advancement of understanding is not a linear one. The biophysics movement of 1847, led by Carl Ludwig, Émil du Bois-Reymond, Hermann von Helmoltz, and Ernst von Brücke, would prove to be the significant "growth spurt" of neuroscience [2]. Their collective efforts to relate biological processes to the fields of chemistry and physics created a new understanding of the neurophysiologic process. Many of their insights and discoveries are still valid, proving the true genius of their work.

The roots of the landmark achievements of Bernstein, Curtis, Cole, Hodgkin, Huxley, and Katz can all be linked to the tour de force of 19th century biophysics. In turn, the contemporary understanding of neuroscience can be attributed to these pioneers, whose work is still considered valid and applicable to the currently accepted models of neurobiology. It is therefore postulated that only marginal advancement in the true understanding of neurological processes can take place without a fair treatment of the history of the science.

II. In the Beginning

The first plenary work on the brain and the suggestion of its function was offered by Galen in 177 C.E. His work with the Barbary ape led to an understanding of many of the physiological processes of the human anatomy, including the brain.

The investigation into the action of the brain would have to wait until the 1600s, when a young philosopher and mathematician named Rene Descartes suggested that the brain (actually the pineal gland) was responsible for controlling the body and mind. He described the body as a machine and the nerves acted as force transducers. Researchers and scientists, such as Leeuwenhoek and Robert Hooke, discoverer of the cell, armed with microscopes and a penchant for discovery, set out to identify the mechanisms of this control.

Among the many contributions Isaac Newton has made to the scientific process, his theory on nerve conduction is the most important to the subject of neuroscience. He believed, as did Borelli and Leeuwenhoek, that nerves were solid and continuous and his thoughts on electrical pulses propagating through nerves had a profound impact on Luigi Galvani, often considered the father of neuroscience. Galvani had used a Leyden jar, a predecessor to the modern capacitor, to store electricity from a lightning storm. He then wired the jar to a severed frog's limb in a dramatic display of the ability of nerves to conduct electricity (figure 1). Though this experiment is not the first of its kind, it is generally considered

to be the first well documented and well executed.

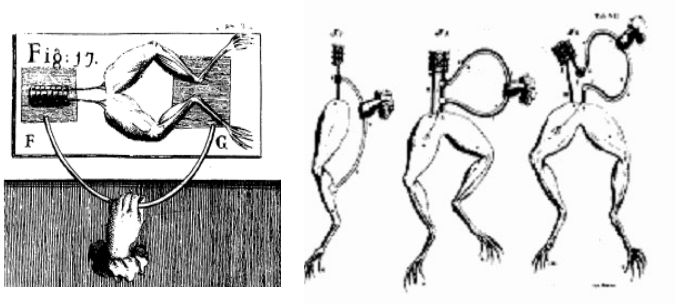

Figure 1. Galvani's frog.

The latter part of the 17th century into the early 18th century spawned a craze for electrical experiments, both for the relationship to the physiological implications as well as the nature of electricity itself. Ewald Von Kleist and Pieter van Musschenbroek devoted much of their time to the development of the Leyden jar. With the ability to store electrical charge, scientists like Alessandro Volta, Charles Augustin de Coulomb, Andre Marie Ampere, and Georg Simon Ohm, set out to describe, in great detail, the mechanisms for the movement of charge [3]. In the midst of this research, two schools of thought emerged; that of an animal electricity and that of a two metal electricity. After Volta succeeded in making the first dc battery from discs of copper and zinc, the idea of animal electricity was suppressed.

In 1831 a professor of Physics at Pisa named Carlo Matteucci used a galvanometer to detect a small electrical current from an injured muscle. Of significant importance, though not realized at the time, was that the current moved from inside the injury to the undamaged muscle surface outside. This single experiment resurrected the work of Galvani and the notion that there was indeed "animal electricity." The search to explain it was afoot.

III. The Golden Age of Biophysics

With the proof of electrophysiological phenomenon came the burden of proving the mechanisms responsible for its existence. In a bold initiative, Hermann von Helmholtz, Émil du Bois-Reymond, and Ernst von Brücke formed an allegiance and adopted as their decree that "*no other forces than common physical chemical ones are active within the organism.*" [4]. Helmholtz, a prominent German physicist, directly measured the speed of a nerve pulse at 100 ft/sec, well within the parameters explainable by electrochemical properties.[4] His collegue du Bois-Reymond had postulated, and later established, that nervous impulses were electrochemical in nature, and traveled much like an electric pulse through a wire. (The comparison of the nerve pulse transduction to that of telecommunications could hardly be discounted and it is likely that the two fields were mutually influential.) He was able to observe a temporary decrease in the "negative variation", the injury current described by Matteucci, though he did not realize the significance of this discovery. He further proposed that muscles contained positive sources and nerves contained negative sinks. Brücke, however, had been approaching the issue of electrochemical properties from the ionic perspective, hypothesizing that osmotic properties were a result of molecular pores. It would be the work of Julius Bernstein, a student of du Bois-Reymond and assistant to Helmholtz, which would help unify the results of Helmholtz, Reymond and Brücke and define the modern understanding of electrochemical transmission. Bernstein would first need to incorporate the seminal work of Walther Nernst.

IV. Diffusion, Gradients, and the Membrane Potential

The work of Wilhelm Ostwald and his artificial semi-permeable membrane established the notion of selective permeability and that the electrical potential seen at these membranes was a result of the permeability of ions [4]. During this time, Walther Nernst, a noted German physicist and chemist, developed the ideas that would eventually lead him to an electrochemical potential formula, known as the Nernst equation. He proposed that the equilibrium potential across a semi-permeable membrane varies in a linear relation ship with the absolute temperature and in a logarithmic relationship with respect to the ionic concentration on either side of the membrane. In algebraic form

$$E_{ion} = \frac{RT}{z_{ion}F} \ln \frac{[ion]_{outside}}{[ion]_{inside}} \qquad (1)$$

where E is the electromotive force, R is the gas constant, equal to 8.3145 V C mol^{-1} K^{-1}, T is the absolute temperature in Kelvin, z is the charge of the ion, and F is Faraday's constant, 9.6485×10^4 C mol^{-1} .

It was shortly after Nernst's publication that Frederick George Donnan, a British chemist, began working on the issue of ionic distribution across a membrane and the resulting equilibrium. He argued that a membrane was selectively permeable and that the product of concentrations of ions that could diffuse through the membrane is proportional on both sides of the membrane. [5] That is

$$\frac{RT}{F} \ln \frac{[ion_1]_{out}}{[ion_1]_{in}} = \frac{RT}{-F} \ln \frac{[ion_2]_{out}}{[ion_2]_{in}}. \qquad (2)$$

By absorbing the negative into the logarithm and inverting the argument of the logarithm, the equation reduces to

$$[ion_1]_{in} \times [ion_2]_{in} = [ion_1]_{out} \times [ion_2]_{out}. \quad (3)$$

This is known as Donnan's Rule and can be applied to systems in finite volume equilibrium [5]. If however an anion that is not permeable with respect to the membrane is present on one side of the membrane, a cation will diffuse through the membrane to maintain electrical neutrality. The presence of this impermeable anion results in an unequal distribution of diffusible ions. The result is a chemical gradient of specific

ions with respect to the two sides of the membrane.

With the identification of the principal ions involved in cellular fluids, namely K^+, Na^+, and Cl^-, Julius Bernstein started with the assumption that muscle and nerve fibers are enveloped by isolating boundary shells (membranes) that are permeable only to specific ions. This was the basis of theory set forth by his childhood friend Ludimar Hermann, who in 1898 proposed his core conductor theory [2]. The theory states that stimulation of the core, which is a conductive core surrounded by a non conductive boundary shell, produces an action current. This action current activates adjacent regions of the fiber through electrical induction. The theory also describes the local excitation in terms of a sudden change or alteration, though Bernstein would later prove this assumption to be false, stating "*One can…with some certainty conclude that a chemical process cannot at all serve as the direct energy source for the electrical energy of muscle current.*" [5]

Bernstein primarily considered K^+ diffusing in the direction of the concentration gradient out of the cell and into the extra cellular fluid. As negative anions, in particular phosphates, apparently could not pass through the cellular boundary, Bernstein argued that an electrical potential builds up across the semi-permeable membrane, which would impede further efflux of potassium. This potential corresponded exactly to the "resting current". Furthermore, Bernstein postulated that an excitation of the fibers would lead to a brief loss of the selective membrane permeability, thereby eliciting "action currents" and a "negative variation" in the potential. Thus, the first postulation of an action potential, and the ability of a cell to elicit an electrical response, was formed.

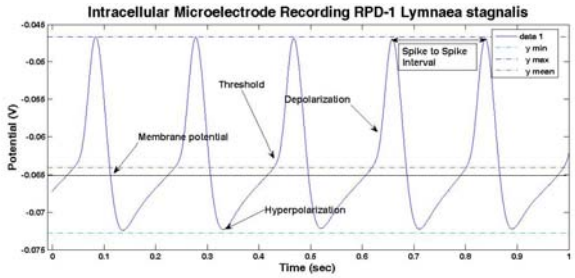

Figure 2. Action potential recording from RPD-1, *Lymnaea stagnalis.*

Bernstein would write in 1902

"*A second assumption* [in addition to the validity of the Nernst equations] *concerning the composition of the concentration chain in muscle is that the electrical potential of the lesioned muscle is caused by the electrolytes, in particular by inorganic salts such as K_2HPO_4, already contained in the undamaged muscle fiber. Let us imagine that these electrolytes diffuse unhindered from the axial cross section of the fibrils into the surrounding fluid, while they are prevented from diffusing through the longitudinal section by an intact sarcoplasmalemma which is impermeable to one kind of ion such as the anion (PO_4 - etc.) to a greater or lesser degree. Then an electrical double layer would emerge at the surface of the fibril, with negative charges towards the inside and positive charges towards the outside. Indeed, this electrical double layer must also exist in the undamaged fiber, but would become apparent only in response to lesion or stimulation (negative variation). This assumption would imply a theory of preexistence; as the semi-permeable membrane plays an essential role in this theory, I will succinctly call it 'Membrane Theory'.*" [6]

V. The Membrane

In 1925, the first accurate glimpse of the membrane was offered by Gorter and Grendel. They showed by empirical means that the cell membrane was composed of a lipid bilayer [2]. By carefully extracting the lipid from a cell, which was performed by dissolving the cell in organic solvent, they were able to isolate only the lipid portion of the cell. They then placed the lipid component in water and observed the lipid float on the surface, as expected. However, what they also observed was that the small polar end of the lipid associated with the highly polar water, while the long nonpolar strands stuck out of the water. Using two wooden floats, they squeezed the edge of the lipid layer together and observed the formation of the lipid bilayer [2].

Danielli and Davson expanded this idea to include the stabilization of this bilayer by a thin layer of protein molecules on both sides of the membrane [7]. This was known as the Davson and Danielli model and served as the accepted model of the cellular membrane for many years. Using a bimolecular layer of lipid 50 angstrom thick with a dielectric constant of 5, the capacitance of the membrane would be about 1μF cm^{-2}, since capacitance is given by $C = \frac{k\varepsilon_0 A}{d}$, where $k\varepsilon_0$ is the permittivity of the lipid, A is area of the membrane in cm^2 , and d is the distance between the layers, approximately 50Å [7].

To develop a full picture of the current carried by each of the principal ions across the cellular membrane, the forces acting on each ion and the permeability and conductance of the membrane to that ion must be elucidated. First, the flux due to electric forces is defined by

$$J_{EMF} = -C\mu z\frac{dV}{dx} \qquad (4)$$

where C is the concentration in moles per cm^3, μ is the mobility of the ion, z is the charge of the ion, and $\frac{dv}{dx}$ represents the change in voltage over the change in distance. There is also a flux due to the chemical forces given by

$$J_{chem} = -\frac{RT}{F}\mu\frac{dC}{dx}. \qquad (5)$$

It is now possible to define the total current across the membrane as the superposition of the two fluxes times Faraday's constant. That is

$$J_{total} = J_{EMF} + J_{chem}$$

$$I_{total} = J_{total} F = -RT\mu \frac{dC}{dx} - C\mu Fz \frac{dV}{dx} \quad (6,7)$$

This is known as the Nernst-Planck flux equation [5]. The term $C\mu F$ is incremental conductance at one point on the membrane. The conductance of the ion over the whole membrane is then just the integration of the sum of each conductance, remembering that conductance in series adds like resistors in parallel.

VI. One Small Step for Neuroscience

The neuron has several major parts: the soma, or body, the dendrite, and the axon. Further divisions in the axon include the axon hillock, the beginning of the axon core, just after the soma, and the presynaptic knob, a bulb like structure at the end of the axon. An action potential is received by a neuron from the dendritic region then processed by the soma before passing some measure of transmission "down" the axon. The axon is typically coated with a myelin sheath that acts as an insulator for the conductive axon. There are, however, gaps, called Nodes of Ranvier, which are sodium channel rich and serve as transmission enhancers. The gap between the axon of one neuron and the dendrite of another is called the synapse. This, of course, is common knowledge now, but in the late 1920s, this was only beginning to come into focus.

Advancements in measurement devices by the early 1930s made it possible to make direct measurements from the squid giant axon, the "conductive cable" portion of the neuron. Two researchers, Howard J. Curtis and Kenneth S. Cole, revisited Hermann's cable theory to help explain their measurements of the membrane impedance. Hermann had suggested that excited regions of an axon adjacent to unexcited regions would generate local circuit currents. He then used the concepts of cable theory to describe the axon as a "leaky" version of a telegraph cable. The Hermann cable representation of an axon consisted of a network of resistors and capacitors in parallel, sandwiched between two resistive rails, representative of the membrane bilayer [2]. Cole and Curtis modeled their membrane with time varying elements to demonstrate that although the capacitance stays constant over time, the conductance and electromotive force vary. (See figure 3)

The time varying component of conductance that Curtis and Cole introduced proved to be extraordinarily significant. They concluded that the increase of conductance begins only after a sharp increase in the membrane potential and that this exponentially rising phase of the membrane potential is the discharging of the local circuits. They would go on to suggest that membrane itself contributed to the net influx of current, though they stopped short of offering an explanation as to which ions might be involved in the process. This is summarized in their 1938 writings as follows:

For these reasons, we shall assume that the membrane resistance and E.M.F. are so intimately related that they should be considered as series elements in the hypothetical equivalent membrane circuit. These two elements may be just different aspects of the same membrane mechanism. [9]

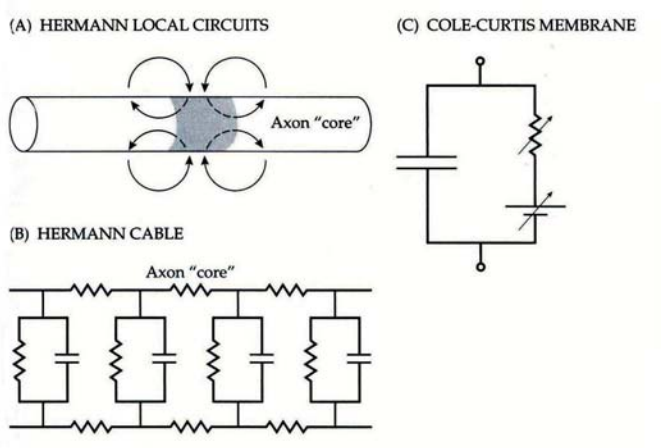

Figure 3. (A) Directional current flow according to Hermann. (B) Hermann's cable core model for the axon. (C) Time varying model proposed by Curtis and Cole. [2]

In the classical image, taken of the front of the oscilloscope, Curtis and Cole illustrated the first direct relationship between an increase in conductance (ion permeability) as a result of increased membrane potential. (figure 4) However, this created more questions than it answered. If the membrane became permeable to all ions, why would the E.M.F of the membrane shoot past 0 mV? This question would lead to the landmark work of Alan Hodgkin, Andrew Huxley, and Bernard Katz, and the publication of a series of papers that is perhaps the most celebrated experimentation for the understanding of the ionic mechanisms responsible for action potential (AP) propagation.

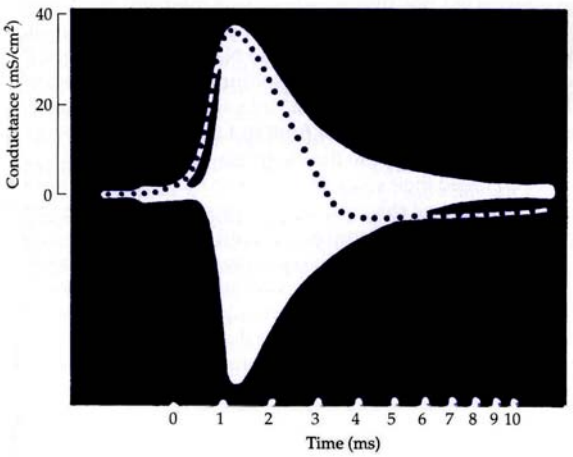

Figure 4 Curtis and Cole's classic picture of the action potential (dotted) and the corresponding rise in membrane conductance.[2]

VII. When the Squid Landed on Plymouth Rock

John Zachary Young was one of the most important biologists of the twentieth century. He was responsible for discovering a bundle of nerve cell bodies in the squid *Loligo* that fused together to form a cable-like fiber core [10]. This core was the squid giant axon and its identification at the Marine Biological Association in Plymouth, UK, spawned a

research boom that would lead to the complete quantitative description of the ionic mechanisms of the action potential.

Young introduced his work to researchers at the Marine Biological Laboratory in Woods Hole, MA, in 1936. Among those researchers were Curtis and Cole. But it was the work of Hogkin, Huxley, and Katz, at the University of Cambridge that would elevate the squid to its place as the most important invertebrate in neuroscience history.

When Alan Hodgkin was still an undergraduate at the University of Cambridge, he was busy conducting experiments to prove Hermann's local circuit theory. He took an axon and laid it on a cold bar, then stimulated it to form an action potential upstream of the cold bar. (The term upstream is used here to denote the fact that action potentials travel in one direction because the refractory period of an AP prevents sodium channels from reopening in response to a change in the membrane potential. This will be covered in more detail later). Because the membrane was chilled at one point, the "voltage-gated" Na^+ channels didn't open there; the membrane could not change its conductance properties and the action potential ceased. But Hodgkin found that even though there was no AP at the cold region, there was one further downstream, beyond the cold bar. He concluded that enough current flowed inside the axon from the point before the cold block to the point after cooled region, that it brought the membrane potential above threshold post cold block, and induced a new AP there [11]. This, however still did not account for the overshoot observed in the action potential beyond the membrane potential

Hodgkin, now working with Bernard Katz, proposed a theory for this overshoot. They hypothesized that a large increase in conductance to sodium and influx down its concentration gradient was responsible for the overshoot. Since the squid giant axon could be rolled out like a tube of toothpaste, Hodgkin and Katz did just that very thing, squeezing the axoplasm and blood from the axon. They measured the concentrations of K^+ and Na^+ and used these concentrations to calculate equilibrium potentials using the Nernst equation. They found that the resulting potential from the K^+ concentration was -75mV while the potential from the Na^+ was 55mV [12]. The sodium theory suggested that potassium was responsible for the resting potential but during the action potential, the membrane became more permeable to sodium, and the maximum potential would tend to the sodium equilibrium potential.

In an elegant series of experiments Hodgkin and Katz showed that perfusion of the axon with sodium deficient solutions in artificial seawater produced action potentials of marked attenuation while sodium rich solutions in artificial seawater produced increased amplitude in the action potential [12]. What they were also able to show is that the rate of change in potential with respect to time was also larger for sodium rich solutions. Given the relationship $I = C\frac{dV}{dt}$, where I is the current and C is the membrane capacitance, the direction of ion flow, i.e., inward or outward, can be determined by the algebraic sign of the derivative. The change in sign of a derivative of a curve is due to an inflexion point and approaches zero as the curve approaches a maximum or minimum. Hodgkin and Katz identified this inflexion point as the point where the membrane becomes permeable to sodium.

Hodgkin and Katz were later joined by A.L. Huxley and together they investigated the current-voltage relationship with a device called a voltage clamp, developed by Cole and Marmont in 1949 (figure 5) [2]. Modifying the equation for current given earlier to include the ionic contribution of current, it can be shown that

$$I = C_m \frac{dV}{dt} + I_{ion} \qquad (8)$$

where I_{ion} is the current due to the ion flow and C_m is the membrane capacitance. When $\frac{dV}{dt}$ is held at 0, that is, the voltage is a constant, or clamped, the current measured is simply the current due to the ion flow. (It is convention to represent outward current flows as positive, or upward deflection, and inward current flows as negative, or downward deflection.)

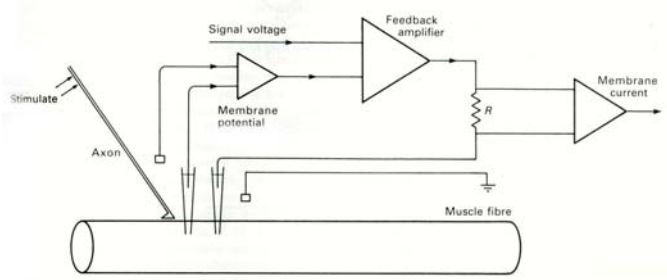

Figure 5. A voltage clamp measures the membrane voltage then passes current back into the membrane to maintain the selected signal voltage.[5]

By manipulating Ohm's Law, $V=IR$, where V is voltage, I is current, and R is resistance, current can be calculated as the potential divided by the resistance. It is equally convenient to define a variable g, which is conductance and is given as the inverse of resistance. Now Ohm's law can be rewritten as $I=gV$. Since conductance is a representation of permeability, an increase in permeability is seen as an increase in current, with the converse also being true. This leads to the set of equations offered by Hodgkin, Huxley, and Katz

$$I_K = g_K(V - V_K), I_{Na} = g_{Na}(V - V_{Na}), I_{Cl} = g_{Cl}(V - V_{Cl}) \qquad (9)$$

where the subscript identifies the individual ion component, g is conductance, V is the equilibrium potential, and V subscript is the equilibrium potential of the specified ion [13].

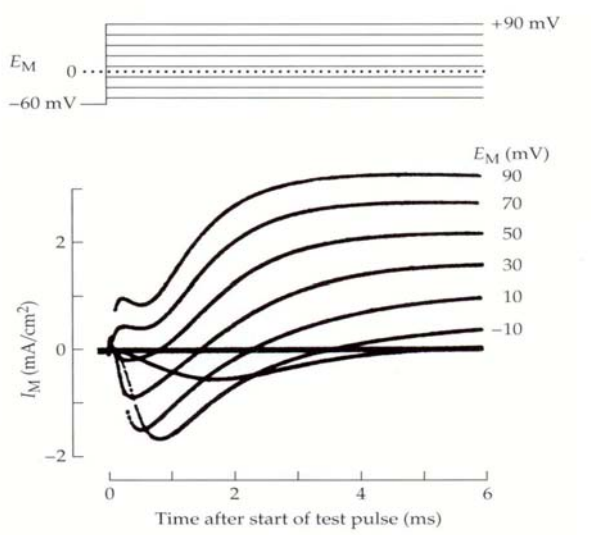

Figure 6. Current response to voltage clamping at varying potentials. [2]

VIII. Active Transport

From the mid 1940s through the 1950s, considerable attention was directed toward the cell membrane and the transport mechanisms responsible for the influx of sodium. Sodium was being ushered into the cell, moving up the electrochemical gradient instead of down. The idea was put forth by WL Dean in 1941 in what he termed the 'sodium pump', but now the search was on to identify how.

Hans Ussing and K. Zerhan had shown in 1950, using the radioactive sodium isotope Na^{24}, that sodium was being actively transported across the membrane. The experiment, which measured the flux, or transport rate, of radioactive sodium across frog skin, proved that the current model of determining the potential difference created by the flux was in need of a correcting factor. They wrote

> *It can be demonstrated …that for a free ion species…the following equation is valid…*
> $\frac{M_{in}}{M_{out}} = \frac{a_o}{a_i} = \frac{c_o}{c_i} \times \frac{f_o}{f_i} \times e^{-\frac{zF}{RT}(\Psi_1 - \Psi_2)}$, *where* M_{in} *means influx and* M_{out} *means outflux…If the solutions on both sides of the membrane are identical*
> $\frac{M_{in}}{M_{out}} = e^{-\frac{zF}{RT}(\Psi_1 - \Psi_2)}$, *in other words:* $\frac{M_{in}}{M_{out}}$ *should be a function of the potential difference only. It is obvious that this equation does not hold for an ion species which is subject to active transport* [14].

There was indeed evidence of a "channel" for ions to pass through. Hodgkin and Huxley expanded these concepts and uncovered the sodium and potassium channels. But it was not just a channel; it actively transported sodium across the membrane. The principal components for the formation of the action potential were unfolding.

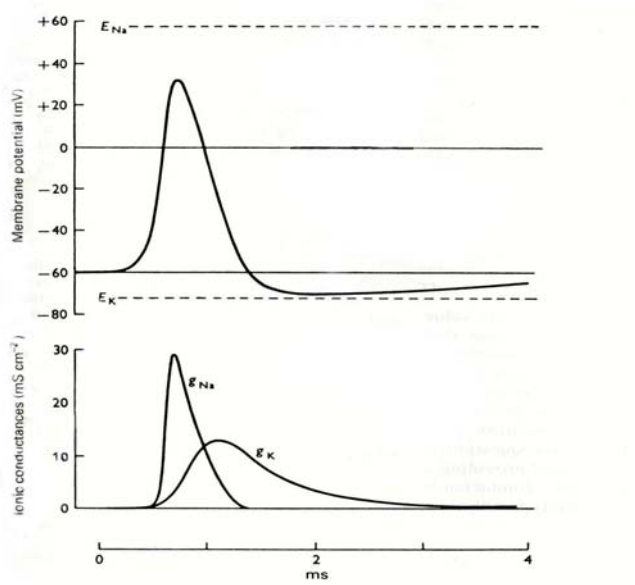

Figure 7. Increased sodium conductance provides initial phase of the action potential, and potassium restores the resting potential.[2]

IX. 'Fat Cats', MEPPS, and Probability

With the identification of most of the mechanisms responsible for action potentials, Bernard Katz teamed up with colleague Paul Fatt to investigate curious fluctuations in the membrane potential of a frog muscle at rest. They had the same shape as an end plate potential, or post synaptic potential, but were much smaller in amplitude, on the order of 0.5mV [15]. Even more curious was the fact that these *mini end plate potentials,* or MEPPs, were caused by the spontaneous release of acetylcholine from the motor nerve ending.

It was well known that end plate potentials were the result of the release of acetylcholine. (This release is triggered by an action potential arrival at the synapse, the region between nerve cells. Regions are defined with respect to the direction of propagation of an action potential. If discussion is of the side before the synapse, that region is described as 'pre synaptic'; otherwise it is 'post synaptic.') Del Castillo and Katz proposed a quantal release theory, as they suspected acetylcholine was being transmitted across the synapse in discrete bundles, also known as quanta [16]. The central theme to this theory was the idea that the release, and arrival, of one packet of acetylcholine (ACh) was responsible for one MEPP and that multiple packets of ACh were responsible for an end plate potential (EPP).

In probability theory, the definition of a single packet with an outcome of success or failure, and nothing in between, is often given by a Poisson distribution. That is

$$f(x) = P(X = x) = \frac{\lambda^x e^{-\lambda}}{x!} \qquad (10)$$

which reads: the probability that the random variable X is exactly equal to some arbitrary value x is the arrival rate λ, or ratio of responses to failures, raised to the x, times the inverse

of the exponential of λ, divided by x factorial [17]. (The distribution function stems from the law of large numbers and is found extensively throughout nature in situations where large numbers of elements, such as ions, are interacting.)

This was a statistical problem concerning the ratio of the number of quanta being released from the pre-synaptic side of the synapse and the number of arrivals at the post-synaptic side. Mathematically, this was a very straightforward problem. The question of interest was how were the molecules of ACh being bundled and released in the first place? And for that matter, how were they being absorbed?

X. Membrane Morphology

Since the days of recess, children in cold winter climates have been daring each other to stick their tongues to metal posts in the dead of winter. The amusement was to watch the struggle of an unwitting child, as he tries desperately to free his subsequently frozen tongue from the pole. What does this have to do with cellular biology? JL Heuser would say, "Plenty!"

In 1979, John Heuser developed a technique, similar to the scenario just described, for separating the lipid bilayer of a cell. By cooling a copper plate to -269°C using liquid helium, he created a "frozen pole" for the cell to stick to (figure 8). He first brought the cell, attached to a similarly cold block, in contact with the copper plate [2]. Then he pulled the block away from the plate, exposing the inside (middle) of the lipid bilayer, not unlike separating a crème filled cookie.

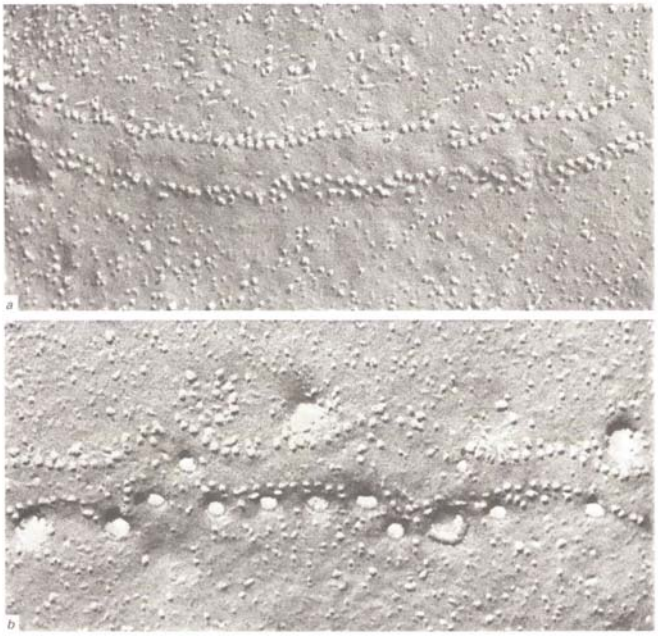

Figure 8. Freeze-fractured image of the separated lipid bilayer. (a) 3 ms before stimulation (b) 5 ms after simulation. The larger openings in b are openings into synaptic vesicles. [5]

The view was dramatic. Heuser had succeeded in exposing the surface of a cell from the inside out. Structures were revealed that had been speculated for years, but now there was proof of their existence. Working with TS Reese and later with TM Miller, he exposed the formation of the vesicle, the quantum packet, as well as a host of other important membrane surface features, including ion channels.

XI. Excitation, Inhibition, and the Synapse

Vertebrates need inhibition. Consider the movement of a sidewinder snake as is scurries across a desert dune. In addition to contracting, or exciting, the muscles on one side of its body in one particular region, it must inhibit the contraction of the muscles on the other side of that region (figure 9). This allows the locomotion to proceed; if one side is not relaxed the other cannot contract. This is the function of EPSPs, excitatory post-synaptic potentials, and IPSPs, inhibitory post synaptic potentials, and their control is mediated by several factors.

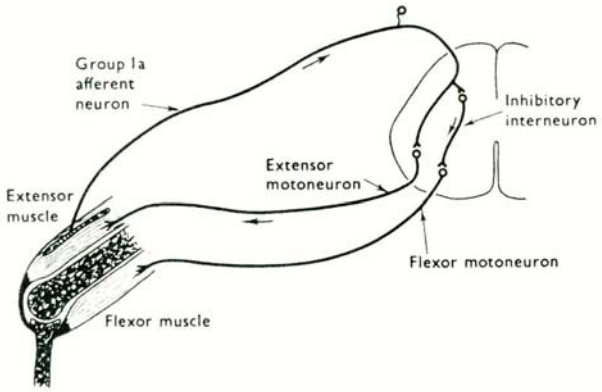

Figure 9. Network of afferent and efferent pathways and inhibitory interneuron to allow opposed muscle groups to work together. [5]

A central component of the mediation of EPSPs and IPSPs is the affect the membrane potential has on them. It is therefore worthwhile to examine the concepts of reversal potential.

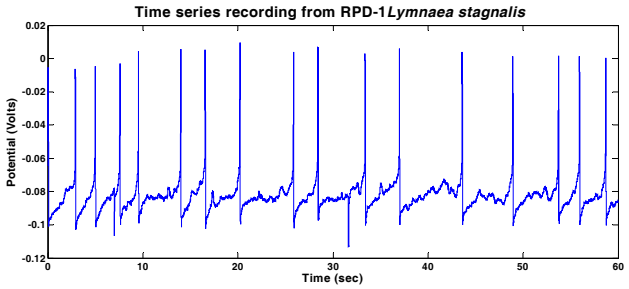

Figure 10. Recording from *L. stagnalis* RPD-1 showing EPSPs. (Small peaks between large peaks)

Hodgkin showed in his experiment using the cold block that small ion fluxes could trigger an action potential. This is significant in that even at zero measurable current, minute ion flux is taking place. The reversal potential (RP) is therefore voltage at which no current flows, even though the channels on the cell surface are open. The RP depends on the concentrations and relative permeability of all the ions involved in generating a current.

Adapting the Nernst equation to accommodate two different types of ions, i.e. sodium and potassium,

$$E_{reversal} = \frac{RT}{zF} \ln \frac{P_{Na}[Na]_{out}}{P_K[K]_{in}} \qquad (11)$$

where R, T, F, and z have their usual values and significance. It is straight forward to see that in the simplified model where only one type (same z number) of ion is present, that when the ratio is less than one, the reversal potential is negative [2]. Thus, the post synaptic membrane is able to identify whether the AP is excitatory, or depolarizing (EPSP) or inhibitory, hyperpolarizing (IPSP). (The IPSP can be depolarizing if the reversal potential for the channel being opened is more negative than the threshold.) The shape of an IPSP is very similar to an EPSP.

JS Coombs showed in 1957 that for an EPSP, the response is proportional to the stimulus intensity. This is known as spatial summation. Whereas if two EPSPs, either of which is too small in intensity to trigger an action potential on their own, are successive in a short time span, the sum of the two can be large enough to trigger an AP. This is known as temporal summation. This is a key mechanism in the decision making process in the post synaptic neuron, since the arrival of a sufficiently large IPSP, within a short enough time span (less than 2 ms) can cancel the effect of an EPSP, i.e. the summation of a depolarized waveform with a hyperpolarized waveform of equal magnitude is zero [5]. The effect of this situation on an end plate potential, EPP, at a motor neuron of a sufficiently large IPSP is to relax the muscle. In this example, the motor neuron is the decision maker, much like a logic gate decides based on the Boolean 'and' or 'or'.

The synapse itself, however, has a morphology that predisposes the type of response, either excitatory or inhibitory. Using electron microscopy, Gray, and later Uchizono, observed shape and thickness differences in known types of synaptic membranes and clefts. Uchizono went on to observe the vesicle itself, the packet containing neurotransmitter, had a distinctive shape, indicative of the type of response they would elicit. Inhibition could be induced at the pre-synaptic region, and was subsequently called presynaptic inhibition.

Still further designations ensued: Type I and Type II synapses, fast and slow synaptic potentials. There were even different physical mechanisms for intercellular communications. Gap junctions were discovered by Ravel and Karnovsky and indicated that cells could communicate without neurotransmitters. This is termed electrically transmitting synapses, or simply electrically coupled synapses. These gap junctions are much larger than a typical ion channel and can accommodate much large ions. This is what enables fast electrical responses that can not be achieved with conventional chemical junctions.

XII. It's a SNAP! (Or a SNARE?)

It has been shown that neurotransmitters can initiate either excitatory or inhibitory postsynaptic potentials. Fatt and Katz discovered that these neurotransmitters were packaged into packets, or quanta. Freeze fracturing and electron microscopy identified these packets as vesicles formed by the cell (see figure 7) The mechanism that determined why and how these vesicles were able to be attached and subsequently released, or alternatively, reabsorbed and recycled, was still unclear.

An action potential induces exocytosis in presynaptic vesicles with the aid of synaptosomal-associated proteins (SNAPs). This is achieved in four steps: 1) the vesicle moves into a region on the presynaptic membrane called the active zone 2) several proteins assist in attaching the vesicle to the active zone 3) a complex of SNARE (SNAp REceptor) proteins attach, or dock, the vesicle to the membrane and 4) rising calcium concentrations in the cell mediates fast fusion of the vesicle to the membrane and the neurotransmitter is released (figure 11) [18].

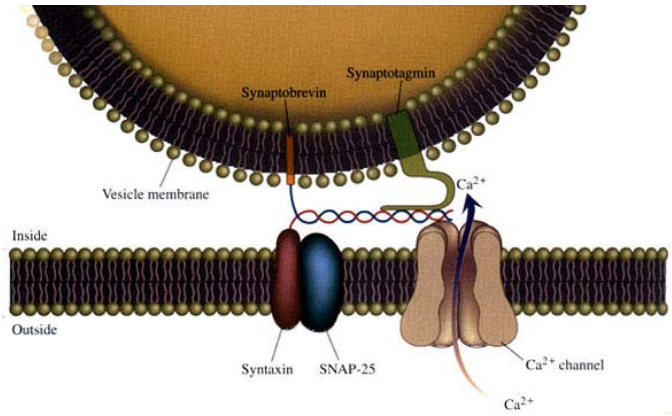

Figure 11. Artist rendition of SNARE complex "docking" the vesicle and the importance of calcium ion to the process. [19]

SNAREs can be categorized as either t-SNAREs, target SNAREs, or v-SNAREs, vesicle SNAREs. Still a further classification names a VAMP, or vesicle associated membrane protein, which is a type of v-SNARE. The SNARE complex of interest is the synaptobrevin (VAMP), syntaxin (t-SNARE), and SNAP-25(t-SNARE) complex. [18]

XIII. Discussion

Neuroscience is more than a science, it is a quest. It is at the heart of human kinds most basic of questions; *what is consciousness*? The history of the science is fraught with exceptionally talented and adventurous researchers, whose collective efforts have labored to produce many of the answers we take for granted today. The majority of researchers presented in this paper are Nobel laureates, as their contributions to mankind are of the highest honor. There are, however, a great many scientists whose names are not listed among the giants of the field not because they did not deserve, but simply because history is kept by those who report *a version* of events. Sir Alan Hodgkin remarked in 1977 that "*...the introduction of the squid giant nerve fibre by J. Z. Young in 1936 did more for neurophysiology and axonology than any other single advance during the past 40*

years" yet often Young is overlooked in the broad strokes of the history of neurophysiology.

It is difficult to imagine where our understanding of thought and behavior would be were it not for the insight of so many great scientists. The action potential, the most basic form of cellular communication, is a simple, elegant waveform, yet the production and propagation of the AP is one of the most comprehensive processes in all of animal life. It transcends the boundary, to a certain degree, between vertebrate and invertebrate and provides more than just a subtle hint that long ago evolution was not quite as diverse as it is today.

By citation of previous works, a history is established that lends credence to the methodologies presented as well as the conclusions that have been offered. It is therefore demonstrated that the research, when taken as a whole, provides a verifiably accurate model of the biological and physiological processes with regard to the electrochemical formation and transmission of communicatory signals.

We now look at sodium, potassium and calcium channels under high powered electron microscopes and reveal a beautifully complicated network of proteins. And the sodium-potassium-ATPase pump, the existence of which was known long before it was seen, continues to intrigue researchers with complexities we are just now beginning to understand. It is exciting to think what the next hundred years will bring.

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