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Preliminary Studies on the Effect of Size on the Action Potential of an Excitable Vesicle

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Abstract: - We present the concept of an excitable vesicle (EV), whose system comprises sodium and potassium ion channels inserted into the membrane of a polymeric vesicle. Set up this way, the EV is predicted to produce an action potential using only the essential elements found in a biological neuron. The EV can be arranged to form many different computational architectures. Minimizing the size of an EV is desirable for the end device, but many issues accompany limiting available internal ions. This paper presents the preliminary findings from deterministic and stochastic Hodgkin-Huxley type models having a limited available internal cell volume. For a single action potential generated from a lone EV, the primary problem associated with shrinking the EV was failure to recover the resting potential. Failure to recover the resting potential occurred below a specific vesicle-size "threshold" for the particular system in this study. Lowering ion channel density helped to lower this threshold. Increasing Na/K pump rate also showed recovery of the resting potential. Using a stochastic formulation resulted in failure to recover resting potential at larger volumes as compared to the deterministic formulation.

Key Words: - Vesicle, ion channel, action potential, electrophysiology, biotechnology, Hodgkin-Huxley, volume effect.

1 Introduction

Biological computation is seen by many in the field of engineering as a viable option for the next platform for calculation beyond the silicon chip. Current ideas for biological computation involve utilizing molecular interactions seen in nature (such as conformational changes in small molecules [1] or DNA sequencing [2]) in place of digital transistors in a modern central processing unit (CPU).

As amazing as the CPU is, it is still a far cry from the complex functions that can be performed by the human brain. However, as humanity gains more insight into neural networks and complex systems, we are getting closer to the possibility of building a man-made machine capable of higher order of computation. The ultimate goal in this case is the construction of an artificial brain.

A single neuron, the smallest physical element of the brain, does not by itself have the capacity to perform complex functions. What aspects of a single neuron allow the complex computations of the brain to emerge? There are many parts to this question, but clearly the dynamic of the communication between neurons is critical [3, 4].

The two main avenues of communication between neurons are: neurotransmitter signaling and direct gap junction signaling. The effective difference between the two modes of communication, of concern to this study, boil down to how fast a signal is propagated. Sherman and Rinzel [5-7] found that a network of cells strongly coupled by gap junctions behave effectively as one super-cell. However, as soon as the coupling is made weaker (a.k.a. lowering the conductance of the gap junction) more complex phenomenon appears. In Sherman and Rinzel's findings, the phenomenon of bursting emerged from a stochastic representation of a network of pancreatic β cells. Is the slower neurotransmitter signaling required to achieve complex behavior? Could we use gap junctions to achieve the same thing if we control the speed at which a signal, namely the action potential, is propagated?

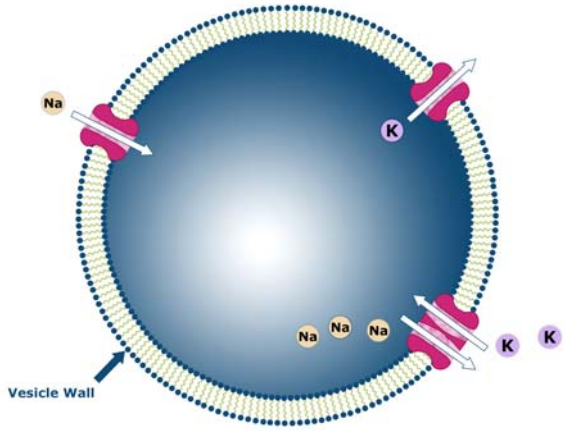
For this study, we focused on gap junction signaling, and more specifically on the dynamics of the action potential. An action potential arises from the flow of charged ions through channels in a cell's membrane.

Instead of studying a whole living cell, we intend to simplify the system to isolate properties that affect the action potential and the communication between cells. The excitable vesicle (EV) system [8] was an ideal tool for this purpose.

1.1 The Excitable Vesicle

The EV is a bio-mimetic polymer vesicle [9] with embedded Na^+ and K^+ ion channels, along with a Na/K pump exchanger (Fig. 1). One of the interesting aspects of the EV is that these channels and pumps may come from completely different types of cells. It follows Hodgkin and Huxley's idea that major contributions to the generation of an action potential are due to the flows of two ionic species, sodium and potassium, across the membrane [10].

Fig. 1: Schematic of the EV system. The excitable vesicle (EV) is a simplified "neuron" where only the sodium and potassium currents are preserved. The Na/K pump attempts to restore the ion concentrations.



Minimizing packaging volume is important to any end device. One aspect of EVs of particular interest to us is what happens to the action potential when the size of the EV is minimized. If the volume is decreased to a point where problems arise from a shortage of ions from the decreased internal volume, the EV system can no longer support its function in the given setup. We address, in the following sections, the effects of the different actions one can take to allow the generation of an action potential in a smaller volume.

We also envision a network of EVs coupled together via gap junctions. Coupled EVs can be organized into several different schemes according to the type of computation to be investigated. One very simple example is to couple three EVs in series as in Fig. 2. The middle vesicle can act like a logic gate or transistor, where the switching criterion is controlled either by taking advantage of ion channel thresholds or the characteristics of the connexin proteins that make up the gap junctions.

The operational mode of gap junction channels is heavily regulated by various properties on both sides of the cell [11]. Connexons (an assembly of 6 connexins) can regulate the passage of ions and cellular signaling molecules less than 1 kD, by opening and closing the channel [12]. Each type of connexin protein forms a channel with a unique voltage-dependant gating mechanism. Furthermore, gap junctions preferentially assemble with neighbors of the same type of connexin [13]. We can exploit both gating and assembling properties to construct a desired network.

Hopfield and Brody [14] describe a more complex neuronal model for spatiotemporal integration. What they term "transient synchrony" refers to the collective transient synchronization of spiking neurons in a voice recognition model. They show a simple neural network hierarchy can exploit synchronization to perform the "many are now approximately equal" operation. Within our network it should be possible to build such a hierarchy by varying physical parameters of the EV. By networking various EVs with connexin, a similar transient synchrony may be realized to perform tasks which require space-time integration.

The approach of using available theory to predict artificial scenarios give us an opportunity to test the

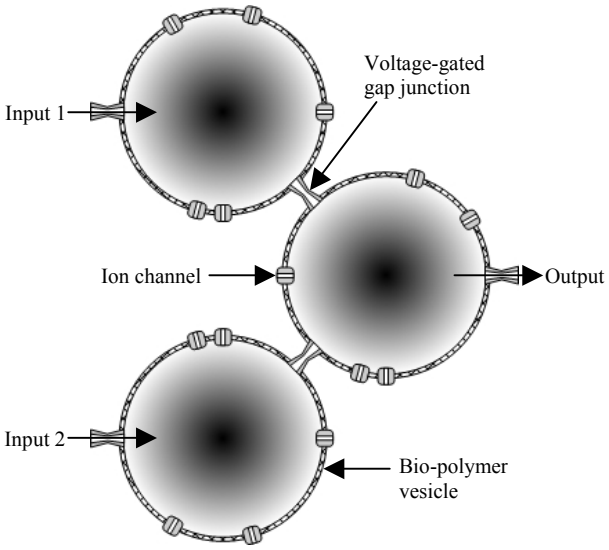


Fig. 2: An example of EVs used as a logic gate. Processing of signals may occur at gap junctions or in the middle vesicle by taking advantage of the thresholds of various ion channels.

robustness of present-day theories. We can construct a new paradigm based on these theories, and then test these new systems by constructing them in the laboratory.

2 Model Description

The models described here were mainly based on the work of Clay and DeFelice [15] and were programmed in FORTRAN90. A stochastic channel gating scheme was applied to the Hodgkin-Huxley formulation of squid giant axon ion channels to simulate single channel activity [16]. Later, a deterministic version of the above model was backed out by replacing the stochastic gating rules with conventional functions using *m*, *h*, and *n* gates (Eqs. 5 and 6). Notable equations are shown below.

Within the models, we also employed an explicit expression for voltage (Eq. 2), as shown by Endresen *et al.* [17], in place of the traditional differential expression (Eq. 1).

$$C_m \frac{dV}{dt} = \sum_x I_x \qquad (1)$$

$$V = \frac{FV}{C_m}\left\{\sum_x [x]_i - [x]_o\right\} \qquad (2)$$

The variables in the above equations were represented as follows: *x* generically denoted the respective ionic species (in our case, only Na^+ and K^+), C_m was the membrane capacitance, *V* was the membrane potential at time *t*, *I* was the ionic current, *F* was the Faraday constant, and

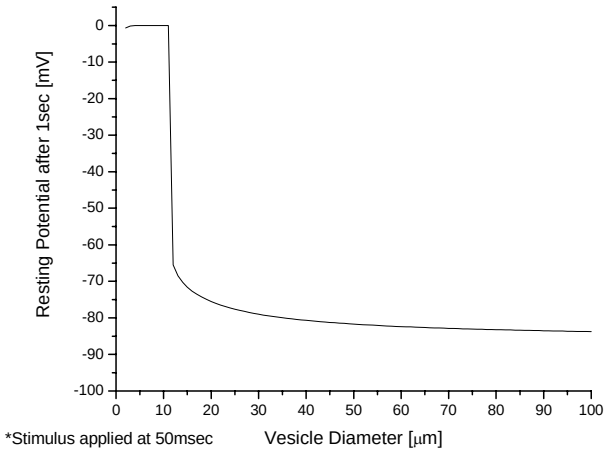
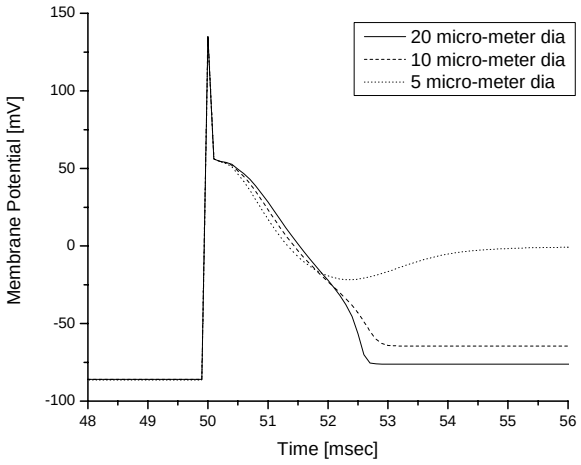


Fig. 4: The failure threshold. Each data point represents resting potentials (RP) sampled at 1000ms, and after a single action potential. For this particular system, there appeared a well-defined region below which the RP was not reestablished. The system was allowed to settle for 50ms before stimulus was applied.

Fig. 3: Shrinking the EV. Smaller vesicles failed to achieve a stable resting potential. Dynamics of the ionic currents failed to drive the membrane potential below the activation thresholds of the ion channels. This example did not include the Na/K pump.



subscripts *i* and *o* referred to the inside and outside of the vesicle.

The changing internal ionic concentrations caused by the limited encapsulated volume of the vesicle were captured by the Nernst potentials (Eqs. 3 and 4),

$$E_{Na} = \frac{RT}{F}\ln\left(\frac{[Na]_o}{[Na]_i}\right) \qquad (3)$$

$$E_K = \frac{RT}{F}\ln\left(\frac{[K]_o}{[K]_i}\right) \qquad (4)$$

where E_{Na} and E_K were the Nernst or ionic reversal potentials for sodium and potassium ions, *R* was the ideal gas constant, and *T* was the absolute temperature. The Nernst potentials were used in the traditional Hodgkin-Huxley current dynamics (Eqs. 5 and 6).

$$I_{Na} = N_{Na} g_{Na} m^3 h(V - E_{Na}) \qquad (5)$$

$$I_K = N_{Ka} g_K n^4 (V - E_K) \qquad (6)$$

Here, *m* represented the activation gate and *h* the inactivation gate for the sodium channel, *n* represented the activation gate for the potassium channel, *N* was the number of channels, and *g* was the single channel conductance.

The Na/K pump formulation followed that of Endresen *et al*. [17] multiplied by a factor of 10 to represent 10 times the number of pumps as there are in a typical patch of a rabbit sinoatrial node cell.

Differential equations were solved using the forward Euler method with a time step of 5ns. This method was chosen due to its simplicity and ability to handle fluctuating variables in the stochastic model.

3 Results

A single action potential was obtained from a system with an applied stimulus current with no leakage of ions added. There was always a small intrinsic leakage of ions in the resting state due to partially open channels. A pacemaker potential was obtained by allowing a sodium leak of 0.3mS/cm^2.

3.1 Effect of Size

As the vesicle diameter was decreased, parameters such as ion channel density and stimulus current were proportionally maintained. The initial potassium concentration inside the vesicle was also modified to achieve a starting membrane potential of -85.9mV. This value was arbitrarily assigned as the average voltage obtained with physiological ion concentrations in a 1μm vesicle.

In Fig. 3, the 5μm vesicle did not come back to a stable resting potential as the larger vesicles have. Instead, the ion gradients were essentially depleted by the action potential event which led to a near zero membrane potential. If the time axis was extended to 1000msec, the resting potential of the 10μm EV would eventually creep to zero.

To investigate whether failure of regaining the resting potential occurred at a specific vesicle size, a trade study was conducted on diameters ranging from 2μm to 100μm (Fig. 4). Stimulus was given at 50msec and the resulting resting potential was sampled at 1000msec. At about a diameter of 11μm, there was a sudden failure of the system represented by the zeroing out of the resting potential.

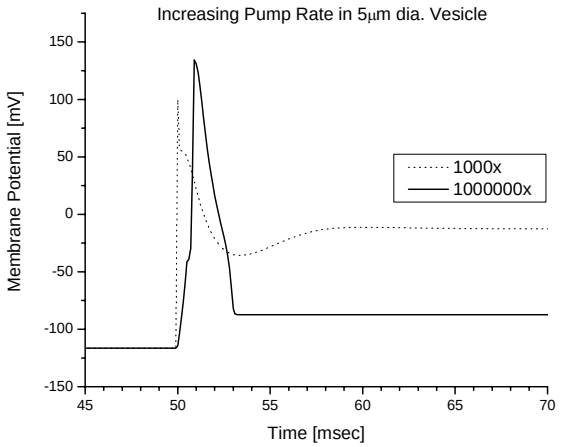


Fig. 5: Increasing pump rate helped to recover the resting potential in a 5μm diameter EV. All other parameters were unchanged. The system was allowed to settle for 50ms before stimulus was applied.

3.1.1 Increasing Pump Rate

In all our models, artificially increasing the overall pump rate produces the same results as increasing the number of Na/K pumps in the system. When the pump rate was increased, the EV showed signs of regaining the ionic concentrations necessary for a resting potential (Fig. 5).

3.1.2 Lowering Ion Channel Density

Lowering ion channel density in the system was simulated by lowering the channel conductances by 20%. Decreasing the conductance of the channels resulted in a lower threshold in which the system fails to regain the resting potential after one stimulus (Fig. 6). The amplitudes of the action potentials remained unchanged.

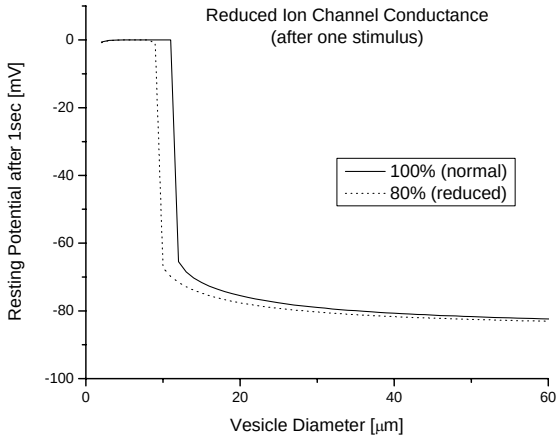


Fig. 6: Effect of reduced ion channel conductance was compared to Fig. 4. Reducing ion channel conductance helped lower the threshold at which the system suddenly failed to regain the resting potential.

4 Discussion

The results presented above show that an action potential is theoretically possible in an artificial environment of relatively small volume. Our goal was to study the different effects that we can utilize to minimize the EV. At this stage in the project, we were more interested in the general phenomena and issues surrounding minimization of an EV. In this discussion, we only make a modest attempt to explain the phenomena presented above.

As stated previously, an interesting aspect of the EV is that these channels and pumps may originate from completely different types of cells. Shrinking the vesicle may be a challenge, but following the trends described above, different components may be chosen to further allow minimization. By comparison to some organelles which generate action potentials, the squid giant axon ion channels used in this study result in relatively large volumes in order to be deemed functional. By looking at the ion channels that populate organelles, we may find further insight into generating an action potential in a small volume.

With respect to how the pump rate affects the system, individual results from each case in Figs. 3 and 4 showed there was very little difference with or without the pump at the baseline pump rate (10 times that of Endresen *et al.* [17]). Fig. 4 showed that when the 10μm case in Fig. 3 was allowed to continue to 1sec, it eventually creeps up to zero potential. The pump appears to begin having an effect when the pump rate approaches 10^6 times the baseline rate (which was 10 times the normal rate found in rabbit SA node cells). This equates to increasing the number of pumps by the same factor. Practically speaking, it is very unlikely that such an enormous number of pumps could fit into the available surface area of an EV.

The relatively lower starting voltage in Fig. 5 was due to the strong pump coming to balance with the ionic currents in the system before the stimulus was applied.

We showed the existence of a threshold in vesicle size where the EV system suddenly fails to return to a stable resting potential. There is some doubt however that this is a true phenomenon. Through the course of our analysis, we have seen resting potentials recovered by merely changing the initial ionic concentrations. Each data point in Fig. 4 required slightly different starting potassium concentration to maintain a uniform starting resting potential for each point. The effect of different starting potassium concentrations could be influencing the result in Fig. 4. There is another fact that might indicate that this is not a true phenomenon. When the system is allowed to oscillate as a pacemaker, only three beats are noticed before the system fails (Fig 7). These facts force us to question whether we have a true resting potential. Future studies shall include applying multiple stimuli to verify the stability of the resting potential.

We shall also compare the results presented here against a different type of model to verify that an important phenomenon such as critical volume is not an artifact of the model.

An in-depth investigation into stochastic effects is planned. Results from earlier analysis (not presented here) showed differences in results between the stochastic and deterministic models at smaller volumes. The models were identical except for the ion channel gating mechanisms. The action potential generated by the deterministic model was able to return to a stable resting potential. However, the action potential generated by the stochastic model using the same parameters and initial conditions failed to return to a stable resting potential.

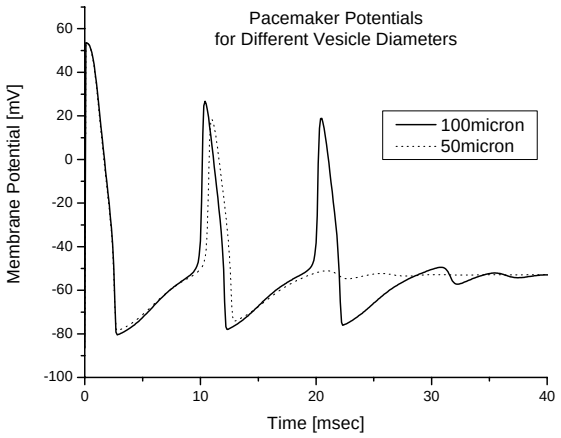


Fig. 7: Effect of size on the pacemaker system. Pacemaker potential failed after just three beats even in a 100μm vesicle. Further decreases in size worsened pacemaker performance. This may indicate the resting potentials shown in Fig. 4 were not stable.

Ionic current fluctuations from the stochastic activity of individual ion channels caused the stochastic EV to fail where the deterministic EV did not. We shall take a detailed look at the regime where deterministic formulations deviate from stochastic formulations. Network properties can also be investigated with stochastic models. We plan on attempting to couple failed stochastically-modeled EVs together to recover function. The emergent bursting phenomenon seen by Sherman and Rinzel may also be investigated.

Our theoretical analysis touches many interesting scientific topics that merit investigation. However, verifying these results with laboratory experimentation is well down the road.

For any hybrid bioengineered system, a sizable gap exists between what is possible in simulations, and what can actually be physically created in the laboratory. The intention is to create a physical EV retaining at least part of the functionality envisioned in this speculation and simulation. Further complicating the task is the need to develop suitable techniques to monitor the function of such unique systems. Ultimately, this may be the limiting factor, as the basic procedures for forming vesicles and incorporating proteins have already been established. For instance, the possibility of using a trans-membrane protein

function in the candidate bio-mimetic polymer has been demonstrated by Choi *et al.* [18] and a prokaryotic potassium channel KvAP was functionally reconstituted by Jiang *et al*. [19]. The experimental challenge now lies in combining these two parts, polymer and ion channels, and determining whether the predicted behavior actually occurs.

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References

[1] R. Adar, Y. Benenson, G. Linshiz, A. Rosner, N. Tishby and E. Shapiro, Stochastic computing with biomolecular automata, *PNAS*, Vol. 101, No. 27, 2004, pp. 9960-9965.

[2] Y. Benenson, R. Adar, T. Paz-Elizur, Z. Livneh and E. Shapiro, DNA molecule provides a computing machine with both data and fuel, *PNAS*, Vol. 100, No. 5, 2003, pp. 2191-2196.

[3] C. Koch, *Biophysics of Computation*, Oxford University Press, 1999.

[4] G. J. Tortora and S. R. Grabowski, *Principles of Anatomy and Physiology (10th Ed.)*, John Wiley & Sons, 2003.

[5] A. Sherman, J. Rinzel and J. Keizer, Emergence of organized bursting in clusters of pancreatic beta-cells by channel sharing, *Biophys. J.*, Vol. 54, No. 3, 1988, pp. 411-425.

[6] A. Sherman and J. Rinzel, Model for synchronization of pancreatic beta-cells by gap junction coupling, *Biophys. J.*, Vol. 59, No. 3, 1991, pp. 547-559.

[7] A. Sherman and J. Rinzel, Rhythmogenic effects of weak electrotonic coupling in neuronal models, *Proc. Natl. Acad. Sci. USA*, Vol. 89, No. 1992, pp. 2471-2474.

[8] C. D. Montemagno, Integrative Technology for the Twenty-First Century, *Ann NY Acad Sci*, Vol. 1013, No. 1, 2004, pp. 38-49.

[9] H.-J. Choi, E. Brooks and C. D. Montemagno, Synthesis and characterization of nanoscale biomimetic polymer vesicles and polymer membranes for bioelectronic applications, *Nanotechnology*, Vol. 16, No. 5, 2005, pp. S143.

[10] A. L. Hodgkin and A. F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve., *J Physiol*, Vol. 117, No. 1952, pp. 500-544.

[11] F. F. Bukauskas, Inducing de novo formation of gap junction channels, *Methods Mol Biol.*, Vol. 154, No. N/A, 2001, pp. 379-393.

[12] Y. Qu and G. Dahl, Function of the voltage gate of gap junction channels: Selective exclusion of molecules, *PNAS*, Vol. 99, No. 2, 2002, pp. 697-702.

[13] A. L. Harris, Emerging issues of connexin channels: biophysics fills the gap, *Q Rev Biopys*, Vol. 34, No. 3, 2001, pp. 325-472.

[14] J. J. Hopfield and C. D. Brody, What is a moment? Transient synchrony as a collective mechanism for spatiotemporal integration, *PNAS*, Vol. 98, No. 3, 2001, pp. 1282-1287.

[15] J. R. Clay and L. J. DeFelice, Relationship between membrane excitability and single channel open-close kinetics, *Biophys J*, Vol. 42, No. 2, 1983, pp. 151-157.

[16] S. Karlin and H. M. Taylor, *A First Course in Stochastic Processes*, Academic Press, 1975.

[17] L. P. Endresen, K. Hall, J. S. Hoye and J. Myrheim, A theory for the membrane potential of living cells, *Eur Biophys J*, Vol. 29, No. 2, 2000, pp. 90-103.

[18] H.-J. Choi, H. Lee and C. D. Montemagno, Toward hybrid proteo-polymeric vesicles generating a photoinduced proton gradient for biofuel cells, *Nanotechnology*, Vol. 16, No. 9, 2005, pp. 1589.

[19] Y. Jiang, V. Ruta, J. Chen, A. Lee and R. MacKinnon, The principle of gating charge movement in a voltage-dependent K+ channel, Vol. 423, No. 6935, 2003, pp. 42-48.