CERTAIN INVESTIGATIONS ON THE GAP JUNCTION CONDUCTANCE IN SYNCHRONIZATION ISSUES

T.S. Murugesh[1*], J. Krishnan[2] & R. Malathi[3]

[1]Assistant Professor, Department of Electronics and Instrumentation Engineering, Annamalai University, Annamalai Nagar, 608002, Tamilnadu, India.

[2, 3] Professor, Department of Electronics and Instrumentation Engineering, Annamalai University, Annamalai Nagar, 608002, Tamilnadu, India.

ABSTRACT

The sinoatrial (SA) node is a thin sheet of cardiac muscle fibers composed of several hundred thousand cells, each of which is an electrical oscillator. Studies of cells isolated enzymatically from the SA node indicate that the intrinsic oscillation frequency of each cell is different. Despite these differences, a coherent oscillatory electrical wave known as the pacemaker potential is generated within the node. This wave is conducted throughout the heart, determining its rate of beating. The pace making cells in the Rabbit heart beat at a wide range of frequencies (80-330 beats per minute) in culture, but within the heart they beat at a common frequency set by the normal sinus rhythm. As a result the synchronization within the heart becomes extremely difficult with such a wide range of intrinsic frequencies. The adjustment of rhythms due to an interaction is the essence of synchronization, the term originating from the Greek words *chronos* meaning time and *syn* meaning the same, common. Upon direct translation "synchronous" means "sharing the common time", "occurring in the same time". This work attempts to explore the issues in the much desired synchronization within the heart with a valid electro physiology model of cardiac pacemaker cells in the cardiac system. For the species Rabbit, an array of sinoatrial node cells was developed and the same was simulated using Matlab software. The simulated results were validated against the prevailing experimental data. The existence of a parameter that can influence the frequency of the so generated action potential was investigated that resembled Gap Junction conductance in real electrophysiology. The intrinsic frequency of the individual cells in the formulated cell array was varied both ways and the resultant effects as observed in the synchronization of the cardiac cells were presented. The functional role of the gap junctions in effecting the much desired synchronization issues within the cardiac system was elucidated.

Keywords: *Sinoatrial Node, Synchronization, Intrinsic Frequency, Action Potential, Gap Junction Conductance*.

1. INTRODUCTION

In the normal cardiac excitation sequence the action potential gets initiated in the sino atrial (SA) node then travels through the atrial wall, the atrio ventricular (AV) node, the Purkinje system and the ventricular wall. The time course of the action potential (membrane potential as a function of time) is notably different in various regions of the heart. When the action potential propagates through the regions, there appear to be cells between the regions that have an intermediate action potential waveform. [1]. It was determined that the cardiac action potential is considerably more complex due to a larger diversity of ion channels present in the cardiac myocyte, the intercellular connections, and its coupling to muscular contraction [2]. In a nutshell, the sinus node is the origin of cardiac activity and generates the contraction orders but the mechanism by which a sinus rhythm is determined is not fully understood [3].

On considering the species 'Rabbit', the Sino Atrial nodal cells have an intrinsic frequency of about 170 beats per minute (bpm), followed by AV nodal cells at 120-330 bpm, and Purkinje fibers at 80-140 bpm. Such a network of cardiac oscillators with a varied range of firing frequencies (330-80 bpm) synchronize and beat at a common frequency corresponding to the normal cardiac rhythm [4, 5]. It has been admitted by the physicians that normally a cell having the highest frequency drives the other cells [6, 7]. But it was suggested that the synchronization among the pacemaker cells plays a crucial role in the determination of the sinus rhythm and its mechanism appears to be more complex [8].

In case of a pair of entrained cells, the time taken to arrive at the entrained state from a random initial condition is to be taken care off. A pair of such cells takes longer time to approach the steady state common frequency if there is a higher difference in its intrinsic frequencies (i.e., time to approach steady state is high if the difference in frequency |f1-f2 | is high) [9]. The problem of rapid return to a stable state, if it occurs at all, need to be more acute in a large

network, like the heart, with a wide range of intrinsic frequencies (330-80 bpm). All the studies pointed out the need for an external parameter that can influence the cardiac oscillators to beat at a single frequency.

2. PROBLEM FORMULATION

Only a small portion of the SAN cells are primary pacemaking cells and they are located in the central part of the SAN of the rabbit. Mathematical models that describe the electrical activity of the Sino Atrial Node (SAN) have been presented by various research groups. In this work we investigate and characterize the effects of the gap junction conductances on the SAN cell array using the Demir et.al. model [10]. With reference to the equivalent circuit for the sarcolemma of the isolated rabbit SAN cell under space-clamp conditions, the differential equation describing the membrane potential (V) is represented as

$$\frac{dV}{dt} = -(I_{Na} + I_{Ca,T} + I_{Ca,L} + I_K + I_f + I_B + I_{NaK} + I_{NaCa} + I_{CaP})/C_m \qquad (1)$$

where I_{Na} is the time and voltage-dependent Na^+ current; $I_{Ca,T}$ and $I_{Ca,L}$ are the time and voltage-dependent "transient" and "long-lasting" Ca^{2+} currents respectively; I_K is the time and voltage-dependent delayed rectifier K^+ current; I_f is the hyperpolarization-activated current ; I_B is the linear background current; I_{NaK} is the electrogenic Na^+-K^+ pump current; I_{NaCa} is the electrogenic Na^+-Ca^{2+} exchanger current; I_{CaP} is the ATP-dependent Ca^{2+} pump current; and C_m is the whole cell membrane capacitance (pico Farads). I_B consists of three linear components: - $I_{B,Na}$, an Na^+ current; $I_{B,Ca}$, a Ca^{2+} current; and $I_{B,K}$ a K^+ current. This model is intended to simulate the electrophysiological responses of a representative transitional cell (the type of cells that surround this primary pacemaking region.) from a region bordering the primary pacemaking region. Details and evaluations of the formulations were referred from the cited model itself [10].

3. PROPAGATION OF ACTION POTENTIAL

All cardiac cells are characterized by continuous contraction and relaxation. Normal contraction depends on normal generation of electrical signals, called action potential, and their organized spread throughout the cardiac tissues. The cells of the cardiac tissues exhibit either excitable or oscillatory system properties. The pacemaker cells are classified under oscillatory system of the cell. All of the heart's cells are capable of acting as a pacemaker cell. The cells located in the sinoatrial node, however, are considered primary because they are faster than the other cells. Normal heart activity gets regulated by waves of excitation generated in the Sino Atrial node (SA node) and propagates through the conducting system and working myocardium. [11]. Pacemaker cells have a specialized cell membrane that allows specific ions such as sodium and potassium to cross and trigger their electrical impulses. Although many these cells can exist in a single location of the body, that too only one cell can fire at a time. Once the first cell generates an electrical impulse a chain reaction is triggered in the other pacemaker cells. This makes them responsible for generating the chain reaction most of the time. The other cells are then secondary and will fire in the event that the pacemaker cells in the sinoatrial node fail to initiate the chain reaction [12].

3.1. Cardiac Networking

The intercellular communication via intercellular low-resistance pathways (gap junctions) and the cardiac networking has become an important subject of research. Cardiac networking addresses the manner by which the cells of the cardiac system are coupled and how do they interact electrically. There are three forms of transfer of excitation: (1) mechanical transmission; (2) chemical transmission, and (3) electrical transmission [13]. The most important transfer mechanism is electrical transmission via low-resistance pathways, which have been identified as gap junction channels. Experiments have proved that before forming gap junction channels, there was no transmission of action potentials from one cell to the other [14]. It can be viewed that the most important mechanism for transmission of excitation is coupling via the gap junction channels. The gap junctions are responsible for the biophysical properties of the tissue. It has been stated that the reduction in gap junction distribution or a closure of the gap junction channels causes non uniformities and discontinuities which alter the biophysical properties of the tissue. The gap junction channel has two main functions: (a) to allow transport of small molecules such as intracellular messengers, small peptides and proteins, nucleotides from one cell to another thereby forming a syncytium and (b) to provide electrical coupling between the cells with or without rectifying properties thereby allowing the propagation of an action potential from one cell to another [15, 16]. Thus, the pore of the channel has to exhibit the much needed properties of an electrical connector that can be turned on and off.

3.2. Distribution of Gap Junctions in the Heart

Heart muscle fibers are coupled by gap junctions. These intercellular channels yield the exchange of small molecules like second messengers, between the cells and they allow electrical coupling. Thus, these cells connected to each other form a syncytium. From the literature it is well known that the conduction velocity varies between 0.3 and 0.6 m/s in the ventricles and 1.0 m/s in the Purkinje system. The conduction is delayed in the AV node too. Also the activation has to be transduced from the sinuatrial node to the atria, and from the endings of the Purkinje fibers to the ventricular myocytes. Hence the coupling within the tissue and between the various cells becomes a critical thing to provide the normal impulse conduction [17]. Gap junction channels span the two adjacent cell membranes and allow the gated transit of molecules from cell to cell. They are formed by a family of proteins, the connexins, which are expressed in most tissues of an organism. Like other membrane channels, gap junction channels too exhibit subconductance states. It seems possible that the subconductance states have a different selective permeability than the full conductance state, so that the flux of larger molecules like second messengers are reduced [18]. Gap junction channels between contacting cells allow the passage of ions and other small molecules between the cells and thereby synchronize cells both electrically and metabolically [19]. Also synchronization of contraction is helped by gap junctional communication as well as synchronization of electrical activation. From the contexts, it can be seen that connecting cells with gap junctions provides both increased speed in synaptic transmission and the ability to synchronize group of cells for coordinated electrical and mechanical output. In addition to electrically excitable cells, virtually all cells in solid tissues are united by gap junctions. A major function of gap-junctional intercellular communication is to share metabolic demands across groups of cells and thereby buffer spatial gradients of nutrients or other signaling molecules [20]. The distribution of the gap junctions between the myocytes is the factor which plays an vital role in the propagation of electrical activity in the heart [21]. Hence from the electro physiology point of view and from the literature studies it can be perceived that the role of gap junctions are important in cardiac muscle; the signal to contract is passed efficiently through gap junctions allowing the heart muscle cells to contract in tandem.

4. CARDIAC CELL – A COMPUATATIONAL MODEL

In cardiac cells many different kinds of ions interact to generate action potential that go through the heart and cause a synchronized normal contraction. Differential equations are developed to define the movement of each ion in the dynamic cardiac environment. On solving all these equations, a general model of the cardiac cell is obtained. The dynamical equations describing the behaviour of a single cell have been solved using Fourth-Order Runge-Kutta method (RK4) and the corresponding source code has been developed using Matlab package. The programs have been executed in a Quad Xeon processor. A single cell model and later on a cell pair model code for a rabbit SA node cell have been initially developed and simulated in Matlab software, with the results matching well with the experimental findings [22].

5. SIMULATION STUDIES AND RESULTS

A cell array was formulated with the cells of the array being coupled with one another by means of coupling conductance that resembled gap junction channels in real electrophysiology. The simulation time and the number of cells that constitute the array were chosen accordingly in order to present an output response with adequate clarity. The parameter (Gap Junction conductance of Sodium ion channel) was maintained at its nominal value for all the members of the cell array and the simulated Action Potential (AP) waveform is shown in Figure 1. It was seen that the SAN cell array was in perfect synchronization with the intrinsic frequency of the cells being maintained at 240 bpm. Here the conductance of the SA node cells was also maintained at a nominal value.

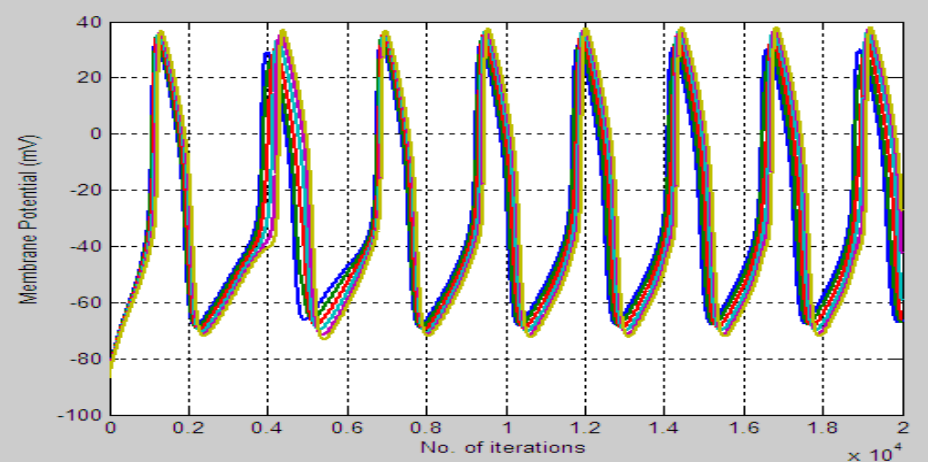

Figure 1. Action Potential of a Rabbit SAN cell array with nominal parameter values

Subsequently the individual parameters of the cells in the array were each provided with a 10% decrease from the nominal value beginning from cell 1 and simulation was carried out. It was observed that the cell array was still in synchronization though with the intrinsic frequencies of the cells being reduced to 210 bpm in contrary to the first case. Interestingly a 10% increase as before also made the cell array to be in synchronization with no changes in their intrinsic frequencies. The conductances of the cells were maintained at its nominal values. The simulated response showed the cells had the ability to regain synchronization but only with a phase difference as shown in Figure 2.

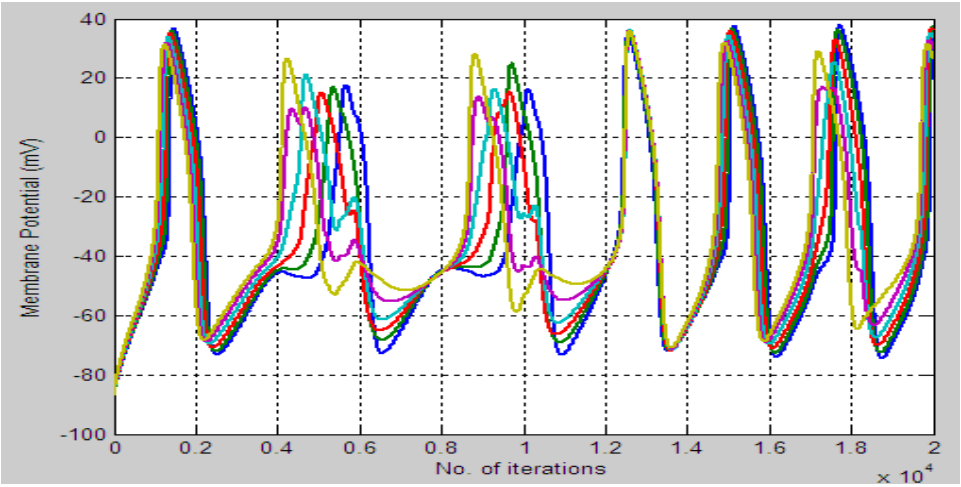

Figure 2. Action Potential of a Rabbit SAN cell array with nominal conductance

For the same changes effected as before the conductance of the cells alone was reduced by 10% from the previous case and the simulation results showed the cells failed to synchronize with the individual intrinsic frequencies of the cells being 210, 240, 270, 210, 180, 180 bpm respectively as shown in Figure 3. For the same changes effected as such in the previous two cases, the conductance of the cells alone was further reduced by 10% and the simulation results showed the cell array failed to synchronize with the intrinsic frequencies of the cells in the array being 270, 270, 210, 180, 120, 60 bpm respectively. The response of the A.P generated is in Figure 4. In both the cases (Figures 3, 4) the cells exhibited a huge phase difference among them.

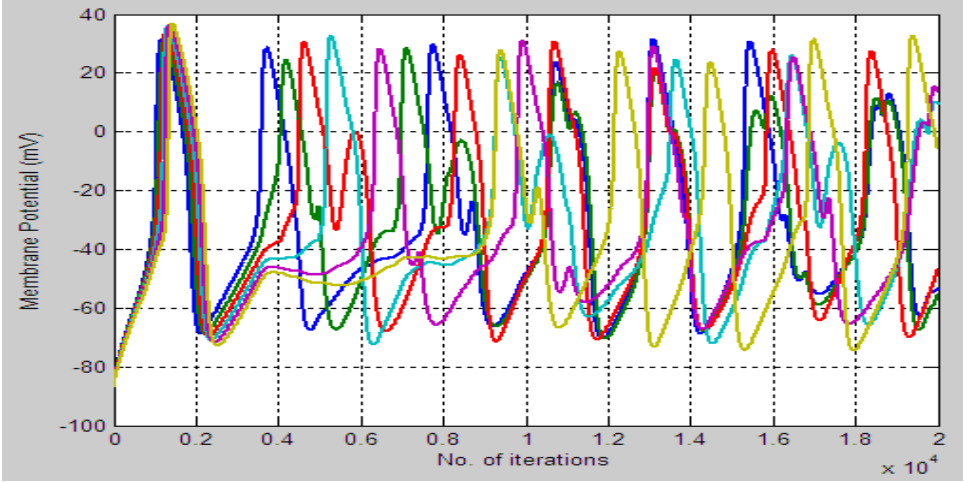

Figure 3. A.P of a Rabbit SAN cell array with lower cell conductance

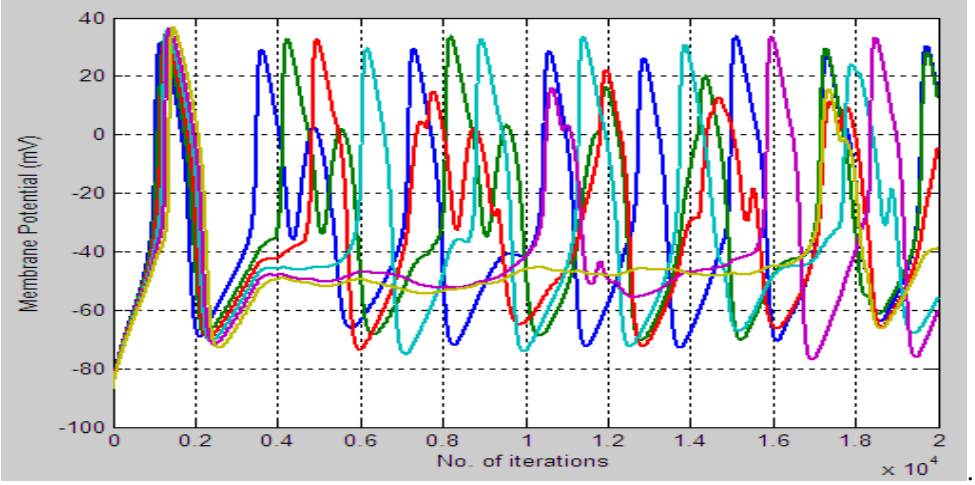

Figure 4. A.P of a Rabbit SAN cell array with lowest cell conductance

From the above studies, it was quite evident that even for a lesser range of reduction (10%) in the conductance of the cells a small change in the parameter of the individual cells can drive the cells away from synchronization. For the same parameter variations the cells with the higher conductance synchronized whereas the results differed for lower values of conductance. Simulation was further carried out to investigate the part played by gap junctions as far as synchronization is concerned. Keeping in mind how the cells will be grouped when formed as a tissue, proportionate changes were made in the individual parameters of the cells in the array whilst the conductance of the cells were maintained constant. It was observed that the cell array perfectly synchronized to a common frequency of 270 bpm without any phase difference as seen in Figure 5.

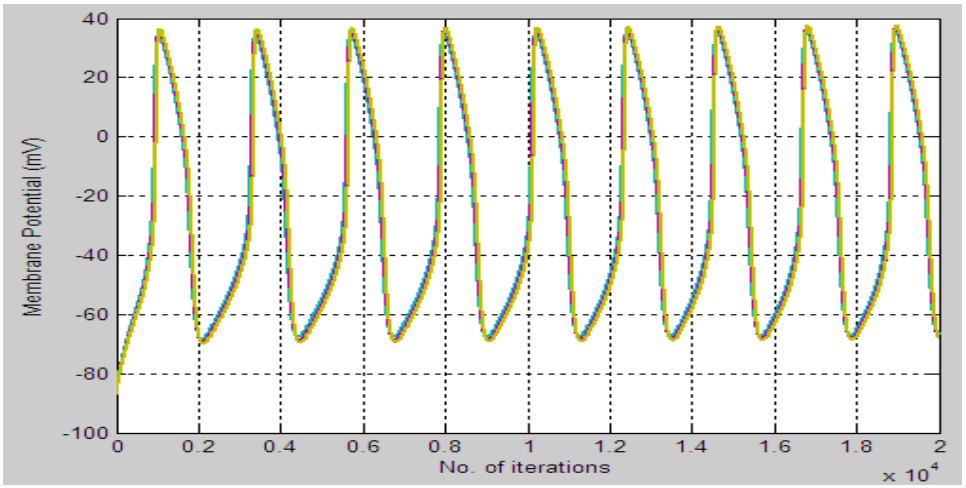

Figure 5. A.P of a Rabbit SAN cell array with varied parameters (lowest conductance)

For the same parameter changes the conductance was increased by 10% and the cell array interestingly synchronized again with the common frequency of 270 bpm but only upon exhibiting a larger phase difference between them as in Figure 6.

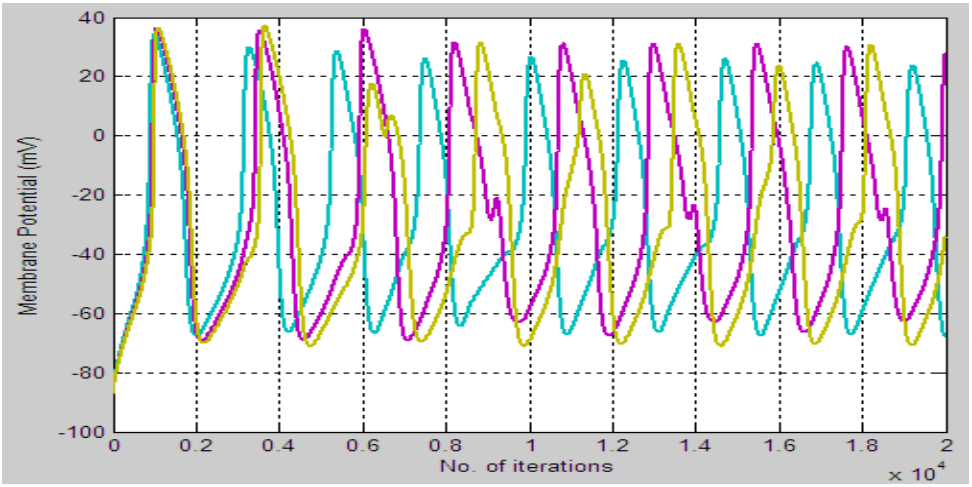

Figure 6. A.P of a Rabbit SAN cell array with varied parameters (increased conductance)

A further increase in the conductance by 10% almost had the cells synchronized in the earlier phase, but ultimately in the end the cell array settled with intrinsic frequencies of 240, 240, 270, 270, 240, 240 bpm respectively and failed to synchronize and exhibited a huge phase difference among them as shown in Figure 7. Now disproportionate parameter changes were made while conductance was maintained intact and it was seen that the cell array again regained their ability to be in synchronization though with a small phase difference between the cells. The simulated response is shown in Figure 8 where the cell array synchronized to a common frequency of 270 bpm.

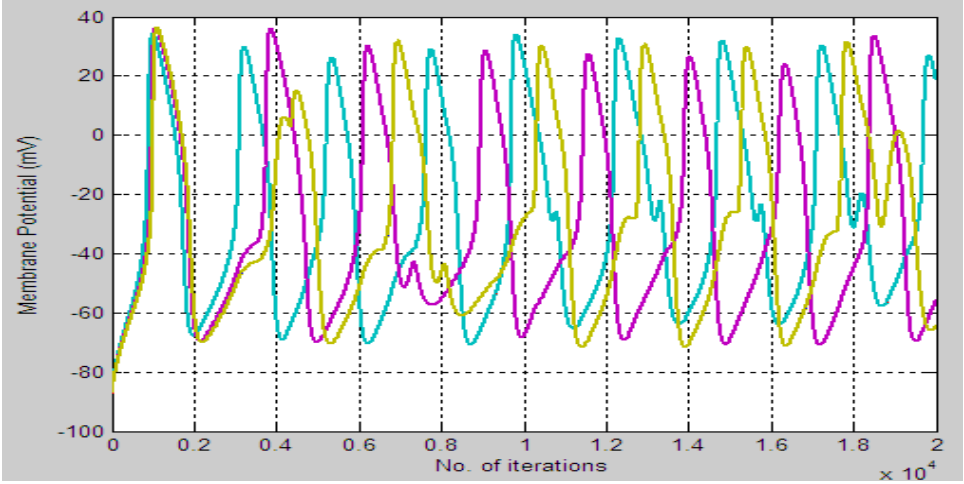

Figure 7. A. P of a Rabbit SAN cell array with varied parameters (highest conductance)

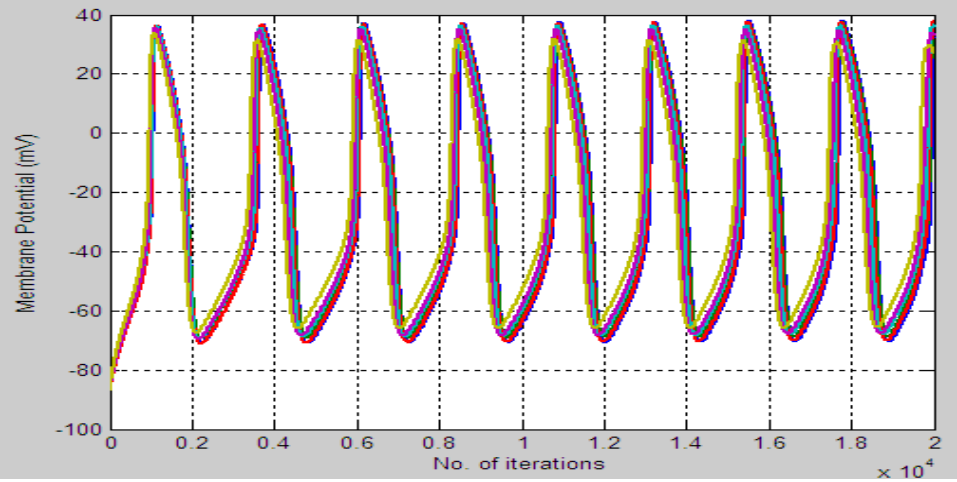

Figure 8. A.P of a Rabbit SAN cell array with disproportionate parameters (lowest conductance)

By maintaining the parameter changes as before an increase in the conductance alone by 10% was made and it was noticed that the cells in the array lose their synchronization and settled with varied intrinsic frequencies of 180, 210, 180, 210, 210, 240 bpm respectively as in Figure 9. Further 10% reduction in the conductance alone worsened the case with the cells having differed intrinsic frequencies of 30, 240, 30, 240, 90, 240 bpm respectively as in Figure 10. It can be observed in the simulated response that the oscillations begin to worn out in some of the members of the cell array.

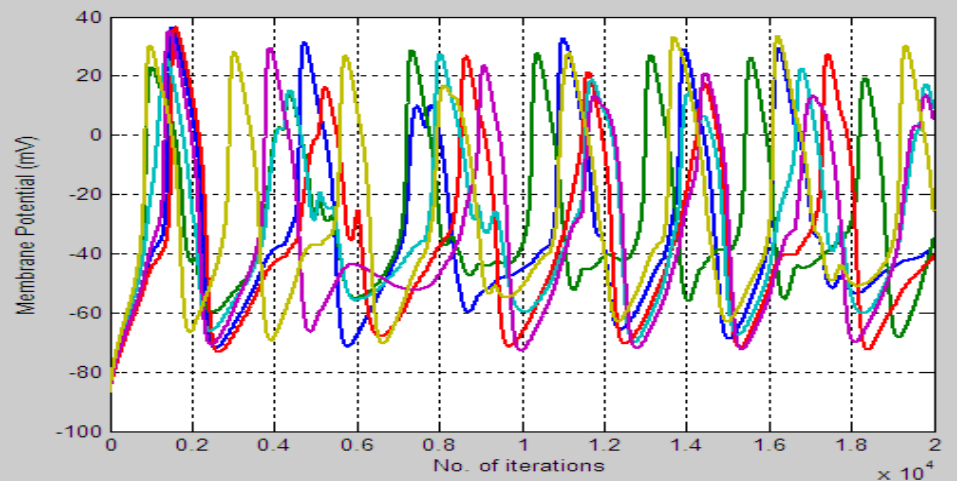

Figure 9. A.P of a Rabbit SAN cell array with disproportionate parameters (increased conductance)

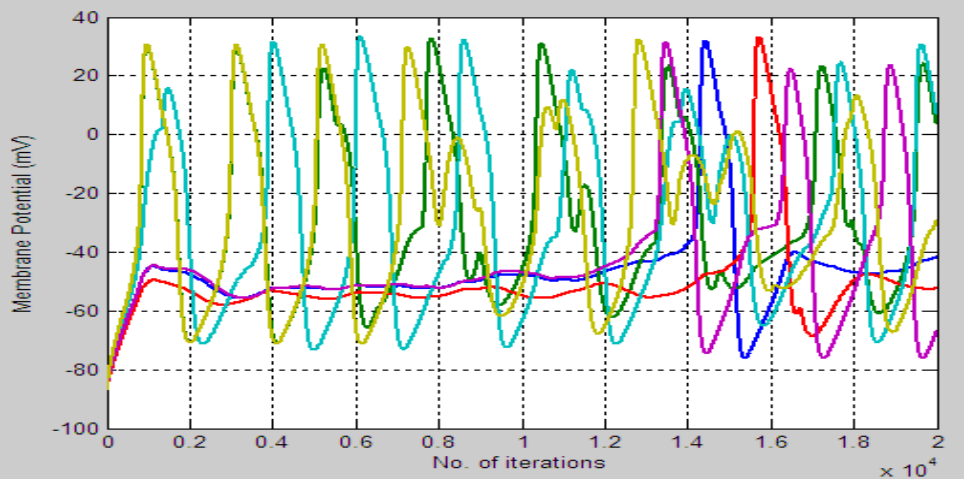

Figure 10. A.P of a Rabbit SAN cell array with disproportionate parameters (highest conductance)

Finally the parameter values that can't make the cells oscillate at all were tried and it was observed that though the cells tend away from synchronization initially, later on they again synchronized to a common frequency of 210 bpm as in Figure 11. It did exhibit a phase difference among them as seen in the simulated response.

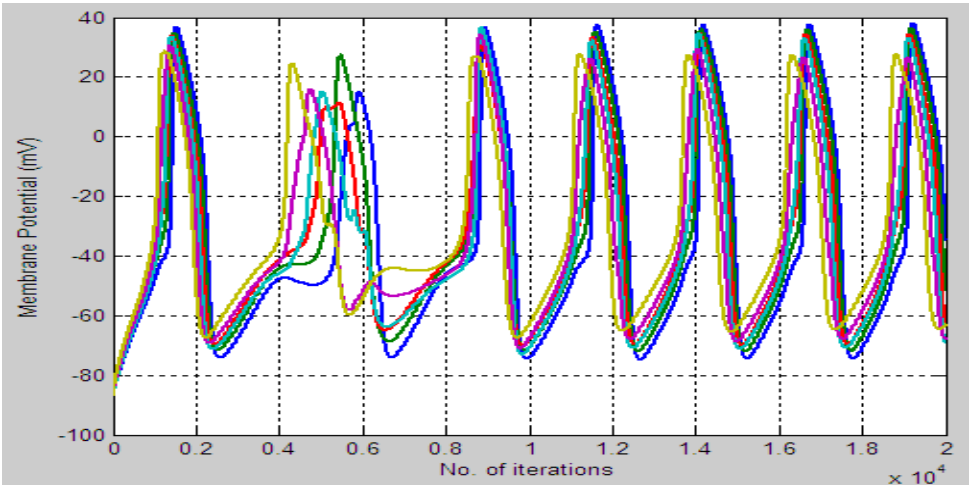

Figure 11. A.P of a Rabbit SAN cell array with large parameter variations (lowest conductance)

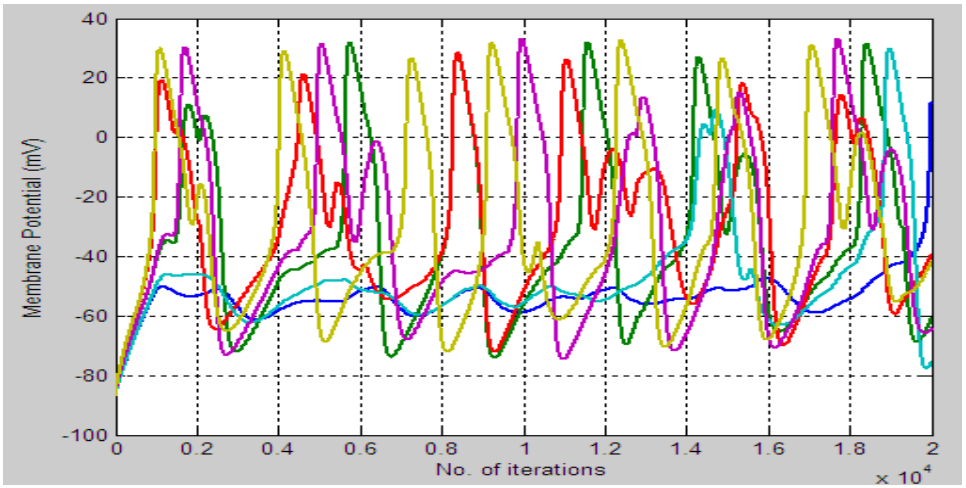

Figure 12. A.P of a Rabbit SAN cell array with large parameter variations (increased conductance)

On increasing the conductance alone by 10% the cell array exhibited a different behaviour with the oscillations beginning to worn out for two of the cells in the array, the cells were no more synchronized and settled to intrinsic frequencies of 30, 210, 210, 60, 180, 240 bpm respectively. The response is seen in Figure 12 with a huge phase difference amongst the cells. A further increase in the conductance by 10% made two of the cells in the array completely non oscillatory, as expected the cell array was not synchronized anymore and settled to intrinsic frequencies of 0, 150, 300, 0, 210, 300 bpm respectively. The simulated response with an unbounded phase difference is shown in Figure 13.

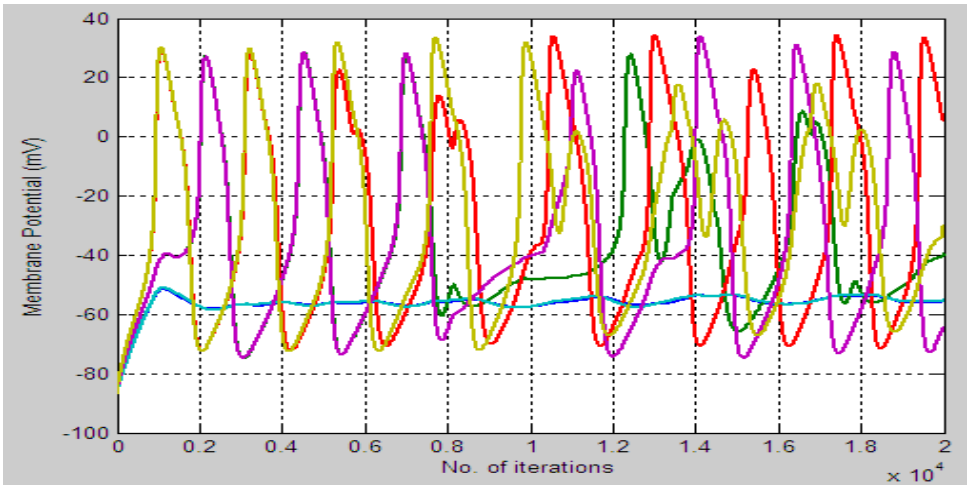

Figure 13. A.P of a Rabbit SAN cell array with large parameter variations (highest conductance)

6. OVERALL CONCLUSIONS

This work attempts to further expound the role of Gap junction conductance's as far as the synchronization issues are concerned. It was inferred from our simulation studies that the variations in the Gap Junction conductance parameters of a cardiac cell array can induce proportionate changes in their intrinsic frequencies. It was understood from the simulation results that higher the gap junction conductance and lesser the difference in intrinsic frequencies between the cells that are part of the array in the cardiac system, better are the chances in regaining synchronization. For a Lesser range of conductance of the cells even a small change in the Gap Junction conductance can coax the cells in the array out of synchronization.

It was noticed from the simulation studies that even upon adjusting the parameter values the cells in the array failed to synchronize as they underwent an unbounded phase difference between them. From the electrophysiology point of view it shows that the value of the gap junction conductance can coax the cells to synchronize only for a limited range of adjustment. Hence the extent to which the gap junctions alone can influence the cardiac cells to be in unison is a point to ponder. Recent findings have also indicated that the ICNS (Intrinsic Cardiac Nervous System)

that can be considered as the little brain in the heart can also play a part in this synchronization issue [23]. Also the recent morphological studies have proposed that the neural influence too can play a part in synchronizing the cardiac oscillators [24]. The research work is being underway to bring out the possible causes that can play a part in influencing the cardiac cells to synchronize in addition to the gap junction conductance.

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