Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 10, pp.3140-3148, October, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INVESTIGATIONS ON THE INTRINSIC FREQUENCY OF THE CARDIAC CELLS IN EFFECTING SYNCHRONIZATION

***Murugesh, T. S., Krishnan, J. and Malathi, R.**

Department of Electronics and Instrumentation Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, 608002, Tamilnadu, India

ARTICLE INFO

Article History:
Received 04th July, 2013
Received in revised form
10th August, 2013
Accepted 25th September, 2013
Published online 23rd October, 2013

Key words:

Cardiac Oscillator
Intrinsic frequency
Sinoatrial node,
Pace maker,
Synchronization
Gap Junction,
Integrate and fire neuron

ABSTRACT

Biological rhythms, like the cardiac rhythm are often generated by large populations of mutually interacting cellular oscillators. The ability of such a population to generate a stable, regulated rhythm depends critically on the nature of interactions among the oscillators, as a network of nonlinear oscillators is intrinsically unstable. Although the entire cardiac system beats in perfect synchronization invivo, intrinsic frequencies of auto rhythmic cells have a significant dispersion. The sinoatrial 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 sino atrial node indicate that the intrinsic frequency of oscillation for 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. This work attempts to explore the issues in the much desired synchronization within the rabbit heart with a valid electro physiological model of cardiac pacemaker cells in the cardiac system. For the species Rabbit, a matlab code for sinoatrial node cell was developed and the simulated results were validated against the prevailing experimental data. The existence of a free parameter that can influence the intrinsic frequency of the so generated action potential was investigated that resembled Gap Junction conductance in real electrophysiology. The functional role of the gap junctions in effecting the much desired synchronization issues with the aid of variations in the intrinsic frequency of the cell within the cardiac system was elucidated and the results indicated that the intrinsic frequency of the cells varied only for a limited range of adjustment. An external neural input was effected via integrate and fire neuron model that further coaxed the cells to oscillate at varied intrinsic frequencies that ascertained the fact that neural influence is much essential to enhance synchronization. This paper investigates with the aid of the simulation results, that the external neural input can also play a part in influencing the intrinsic frequency of the cardiac cells thereby effecting synchronization.

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 (Joyner and Van Capalle 1986). It was determined that the cardiac action potential is considerably more complex due to a larger diversity of ion channels present in the cardiac myocytes, the intercellular connections, and its coupling to muscular contraction (Bin He 2004). 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 (Ikeda Noriaki 1982). 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 (Trautwein and Uchizono 1963; Denyer and Brown 1980). It has been admitted by the physicians that normally a cell having the highest frequency drives the other cells (Harary and Farley 1963; Goshima 1969). But it was suggested that the

***Corresponding author: Murugesh, T. S.,** Department of Electronics and Instrumentation Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar, 608002, Tamilnadu, India.*

synchronization among the pacemaker cells plays a crucial role in the determination of the sinus rhythm and its mechanism appears to be more complex (Sano *et al*., 1978). 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) (Strogatz 1994). 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 rabbit 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.

Synchronization

Mechanism of Synchronization

Synchronization as the word suggests means to bring together, occur simultaneously or be in harmony. The textual translation is "sharing the common time" or "occurring at the same time". The SA node that serves as the normal pacemaker of the heart is comprised of thousands of cells whose intrinsic frequencies are not identical (Bleeker *et al*., 1980; West and Belardinelli 1985). The electrical activities of the various SA node cells are coordinated to give rise to a single impulse by means of a complex mechanism during each cardiac cycle. It was shown that, when aggregates with different spontaneous frequencies are brought into close opposition, their activities synchronize to a common frequency. Further studies indicated that this behavior was related to the time-dependent formation of gap junctions between opposed aggregates that supported the contention that synchronization is mediated by electrotonic influences between clusters that require the formation of gap junctions (DeHaan 1982). However the electrical events and dynamic interactions that were necessary to achieve and/or maintain such synchrony were less explained. From the Literature it was evident that SA node cells are electrically coupled via low-resistance pathways (Bonke 1973; Bukauskas *et al*., 1977; Bleeker *et al*., 1980). Therefore it is possible that electrical coupling forms the basis for the coordination of automatic activity of neighboring cells. Furthermore, experimental results indicate that electrotonic currents traveling in either direction from one pacemaker cell to another can alter the duration of their respective periods (Jalife 1984). These mutual interactions are phase dependent and can prolong or abbreviate the pacemaker's discharges so that synchronous firing can be achieved. Thus, it has been suggested that mutual interactions (i.e., mutual entrainment) may be the mechanism by which the spontaneous firing of individual cells or groups of cells in the SA node is coordinated to initiate the heart beat (Delmar *et al*., 1986; Jalife 1984; Winfree 1980).

Cardiac muscle as a Syncytium

Cardiac muscle fibers are made up of many individual cells connected in series and in parallel with one another. At each intercalated discs (that are actually cell membranes that separate individual cardiac muscle cells from one another) the cell membranes fuse with one another in such a way that they form permeable "communicating" junctions (gap junctions) that allow almost totally free diffusion of ions. Therefore, from a functional point of view, ions move with ease in the intracellular fluid along the longitudinal axes of the cardiac muscle fibers, so that action potentials travel easily from one cardiac muscle cell to the next, past the intercalated discs. Thus, cardiac muscle is a *syncytium* of many heart muscle cells in which the cardiac cells are so interconnected that when one of these cells becomes excited, the action potential spreads to all of them, spreading from cell to cell throughout the latticework interconnections. The division of the heart muscle into two functional syncytiums, *atrial syncytium* and *ventricular syncytium* allows the atria to contract a short time ahead of ventricular contraction, which is important for the effectiveness of heart pumping (Guyton and Hall 2007).

Model development

Experimentally based models of the heart have been developed beginning with the discovery and modeling of potassium channels. The early models were based on extensions of Hodgkin Huxley nerve impulse equations (Noble 2007). There are many more ion channels and other transporters than being cited in the early models. Throughout all living cells there is a broad array of charged atoms called ions interacting at a dynamic level. Ion channels along cell membranes open and close to allow interactions. In cardiac cells, for instance, many different kinds of ions interact to generate action potential that go through the heart and cause a synchronized normal contraction. The electrical activity of the heart is caused by the opening and closing of ionic channels in the cardiac membrane. The ions provide inorganic chemicals for cellular reactions. Also they are essential for some specific cellular control mechanism (Guyton and Hall 2007). 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.

Development of Cardiac Cell Models

In a normally functioning cell, several ionic currents contribute to the changes in the transmembrane potential. Among all the most important ions are sodium, potassium and calcium. A model for the total ionic current across the membrane may be constructed by describing each current with an expression of the form and combining these expressions to form a model for the total current. The first model of this kind was that of Hodgkin and Huxley which was published in 1952 which describes the action potential of a squid giant axon, a particularly large nerve cell found in squid. Hodgkin-Huxley showed in their work how a nonlinear empirical model of the membrane processes could be constructed. The series of 5 papers from them was aimed at determining the laws that govern the movement of ions during electrical activity. Their work described the time course of the current that flows through the membrane of the squid giant axon when the potential difference across the membrane is suddenly changed from its resting value and held at the new level by means of a feedback circuit using voltage clamp techniques. Their work was mainly concerned with the identity of the ions that carry the various phases of the membrane current (Noble *et al*., 2012). The H-H model was so elegant and unprecedented in the quantitative and complete nature of its description that it provided an intellectual framework for biophysical and

modeling work that would influence the field for decades. Moreover, their collaboration exemplified a balance between experiment and theory that has rarely been matched (Hausser 2000). Even today, most biophysical spiking models are based on the H-H equations but the shape of the action potential substantially differed from that of cardiac cells. To obtain physiologically meaningful results, the models that give a more accurate description of the behavior of heart cells need to be considered. Thereby it is attractive to use models with a more detailed representation of the underlying cellular physiology.

Other Cardiac Cell Models

There exist many more ion channels and other transporters than cited in the earlier Hodgkin-Huxley (HH) models. The Noble model was a successful attempt to simulate the action potential of Purkinje fibers with a simple model of Hodgkin-Huxley type. The Noble model is still very similar to the HH model, in that it describes a transmembrane current that carries two different ions: sodium and potassium. The passive leakage current present in the Hodgkin-Huxley model is assumed to be zero in the Noble model (Noble 1962). The aim of the computations described in the Noble model was to test whether, with certain modifications, Hodgkin & Huxley's formulation of the properties of excitable membranes may also be used to describe the long lasting action and pace-maker potentials of the Purkinje fibres of the heart. However, because the model was created before detailed data on ionic currents in cardiac cells became available, the underlying physiology is incorrect. An improvement of the model includes more ionic currents, and gives a much more accurate description of the underlying activity of different channels than the original model. The extension of the voltage clamp technique to the heart revealed a much richer array of ion channels, including multiple delayed rectification channels, calcium channels and the involvement of the sodium–potassium and sodium–calcium exchangers. The MNT (McAllister–Noble–Tsien) model added the multiple slow potassium ion channels and the calcium ion channels. These were significant extensions in themselves, but the importance of the MNT model is that it was the first to use detailed experimental measurements for deriving the voltage dependence of the rate equations in the HH formulation (McAllister *et al*., 1975). The influence of external sodium on action potentials, pacemaker activity and intracellular sodium were also showed in the DN (DiFrancesco-Noble) model. The ionic current changes due to the Na-K pump have been explained extensively. This also was a major extension in terms of types of ion channels and it opened the way to more accurate models of pacemaker activity. There was, however, an additional aspect which was ground-breaking. This was the incorporation for the first time of changes in intracellular and extracellular ionic concentrations and of the intracellular calcium signaling system. This was the most significant departure from the HH formulation (DiFrancesco and Noble 1985). Cardiac models continued to be inspired by the Hodgkin–Huxley work, but they also started to include many processes that were not in the HH nerve equations.

Demir Model

An improved mathematical model of the pacemaker cell from the rabbit SAN on the basis of quantitative whole cell voltage clamp data from enzymatically isolated single SAN cells was proposed by S.S. Demir, J.W. Clark, C.R. Murphey, and W.R. Giles (Demir *et al*., 1994). The model includes intracellular Ca^{2+} buffering and Ca^{2+} uptake and release by the sarcoplasmic reticulum that acted in the same way as HN model (Hilgemann and Noble 1987). The features of the model include, new equations for the hyper polarization activated inward current, assessment of the role of the transient type Ca^{2+} current during pacemaker depolarization, inclusion of a Na^+ current based on experimental data and demonstration of the possible influence of pump and exchanger currents and background currents on the pacemaker rate. With the aid of equations the ion channels Na^+-K^+ and Ca^{2+} pumps and Na^+-Ca^{2+} exchanger in the sarcolemma (surface membrane) are described in this model. The differential equation describing the membrane potential (V) is represented as

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

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: $I_{B,Na}$, a Na^+ current; $I_{B,Ca}$, a Ca^{2+} current; and $I_{B,K}$ a K^+ current. Material balance expressions are provided for the ionic species (Na^+, K^+, Ca^{2+}) in the intracellular medium with equations describing the intracellular Ca^{2+} binding to specific myplasmic proteins, calmodulin and troponin in this model. The cardiac cell is surrounded by a membrane **(**plasmalemma **or** sarcolemma) with unique properties that allow the origination and then the conduction of an electrical signal through the heart leading to near-synchronous depolarization of atrial myocytes and, with an appropriate delay, near-synchronous depolarization of ventricular myocytes. The sarcolemma further possesses properties that lead to the initiation of the excitation-contraction coupling process. Finally, the sarcolemma allows regulation of excitation, contraction, and intracellular metabolism in response to neuronal and chemical stimulation (Opie 1991). The recorded whole cell voltage clamp data allowed more accurate simulations of the spontaneous activity of the SAN cell than was possible with earlier models. The Demir *et al*., 1994 model provided acceptable fits to voltage clamp and action potential data and can be used to seek biophysically based explanations of the electrophysiological activity in the rabbit SA node cell (Hilgemann and Noble 1987) and the same model is chosen for this investigation studies. One more advantage of the chosen model includes the existence of a free parameter that resembles “gap junction conductance” in real electro physiology. Details and evaluations of the formulations were referred from the cited model itself.

Cardiac Networking

Gap Junctions

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 (Sperelakis 1979). 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 (Weingart and Maurer 1988). 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 (Simpson *et al*., 1977; Schwarzmann *et al*., 1981).

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. The coupling within the tissue and between the various cells becomes a critical thing to provide the normal impulse conduction (Dhein 1998). 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 fluxes of larger molecules like second messengers are reduced (Yang and Dahl 2002). 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 (Bruzzone *et al*., 1996). 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 (Goodenough and Paul 2009). The distribution of the gap junctions between the myocytes is the factor which plays a vital role in the propagation of electrical activity in the heart (Dhein 1998). 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 (Chadwick and Goode 2005; Murugesh *et al*., 2012; Murugesh *et al*., 2013).

Biological Neuron Physiology

The firing of a neuron, referred to as the action potential, is an all or none response. This means that, incoming stimuli either produce action potentials, if they exceed the neuron's threshold value, or they do not. A spike or action potential is a stereotyped impulse of fixed magnitude generated by the neuron. After the firing of an action potential, the neuron enters a refractory period when no further action potentials can be generated. Even with very strong input, it is impossible to excite a second spike during or immediately after a first one. This causes that action potentials in a spike train are usually well separated. The minimal distance between two spikes defines the absolute refractory period of the neuron. The absolute refractory period is followed by a phase of relative refractoriness where it is difficult, but not impossible, to generate an action potential (Kandel *et al*., 2000; Gerstner and Kistler 2002). When an action potential arrives at a synapse, it triggers a complex set of biochemical processes that lead to a release of neurotransmitters from the presynaptic terminal into the synaptic gap. The voltage response of the postsynaptic neuron to a presynaptic action potential is referred to as the postsynaptic potential. A single synaptic input is rarely sufficient to determine the generation of an action potential. This is usually triggered as a consequence of the nonlinear interaction of several excitatory and inhibitory synaptic inputs (Mel 1993; Poirazi *et al*., 2003a; Poirazi *et al*., 2003b). At the end of the synaptic action, the postsynaptic potential decays exponentially towards the resting value with a decay rate of its time constant.

Integrate and Fire Neuron

Neurons have remarkable numbers of shapes, sizes, and functions, and therefore also exhibit many different types of dynamics. The simplest is called "integrate and fire:" The neuron's membrane potential rises as the result of stimulation from other neurons until it reaches a threshold following a simple forced ordinary differential equation. Once it crosses the threshold, the membrane potential forms a brief but intense "spike" and then gets reset to its initial value from which it begins rising again. The spike, also known as the "action potential" provides a stimulus to neighboring neurons via a complicated electro-chemical process called synaptic transmission. (Kovacic 2001) The integrate-and-fire neuron model has become widely accepted as one of the canonical models for the study of neural systems. The model provides a good description of the subthreshold integration of synaptic inputs, which occurs on a time scale that is slow in comparison to the rapid spike generation (Burkitt (August) 2006). The state of the neuron is characterized by its membrane potential described in terms of the synaptic inputs and the injected current that it receives. An action potential (spike) is generated when the membrane potential reaches a threshold, but the actual changes associated with the membrane voltage and conductances driving the action potential do not form part of the model. The synaptic inputs to the neuron are considered to be stochastic (Burkitt 2006). The membrane potential receives excitatory or inhibitory contributions by synaptic inputs that arrive from other neurons by their associated synapses. These

inputs that are each weighted by their respective synaptic strength are modeled either as injected current (current synapse models in which summation is linear) or as a change in the membrane conductance (conductance synapse models in which summation of the synaptic input is nonlinear, i.e., the amplitude depends upon the value of the membrane potential). The integrate-and-fire neuron model is a point neuron (single compartment) model in which the spatial structure of the neuron associated with the dendrites is neglected. The neuron is leaky since the summed contributions to the membrane potential decay with a characteristic time constant (the membrane time constant). If this decay of the membrane potential over time is neglected, the model is a perfect integrator (Gerstein *et al*., 1964). When the membrane potential reaches a (fixed) threshold, an output spike is generated - the integrate-and-fire mechanism. After the membrane potential crosses threshold it is reset to its resting value and is inactivated for a brief time related to the absolute refractory period of the neuron. The model is described by the dynamics of the neuron's membrane potential, *v(t)*,

$$C_m\, dv(t)/dt = I_{leak}\,(t) + I_s\,(t) + I_{inj}\,(t) \qquad (2)$$

where C_m is the membrane capacitance, $I_{leak}\,(t)$ is the current due to the passive leak of the membrane, $I_s\,(t)$ is a current describing the effect of synaptic input to the neuron, and $I_{inj}\,(t)$ is a current injected into the neuron (by an intracellular electrode). The leak current is

$$I_{leak}\,(t) = -\,C_m/\tau_m\,(v(t)-V_0) \qquad (3)$$

where V_0 is the resting potential and τ_m is the passive membrane time constant, which is related by $\tau_m = R_mC_m$ to the capacitance and the leak resistance R_m of the membrane potential, both assumed constants (Burkitt 2006). The Autonomic Nervous System (ANS) can be thought of as the regulatory system that partly or wholly controls most of the body's organ systems and homeostatic mechanisms. In general, ANS effects are involuntary, relatively rapid, neuronal reflexes. The specific role of the ANS includes the cardiac output control. From the physiology it is perceived that the sympathetic stimulation increases the heart rate whereas the parasympathetic stimulation decreases it (Pratt *et al*., 2005). The developed integrate and fire neuron model can be thought of to emulate the same functions of the ANS to play its role in cardiac output control.

Cardiac Cell – A computational 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 for the model considered. On solving all these equations, a general model of the cardiac cell is obtained. The dynamical equations describing the behaviour of a single SAN 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 as well as a cell pair model code for a rabbit SA node has been initially developed (Murugesh *et al*., 2012) and was later on extended to an array of SA cells and the same was simulated in Matlab software, with the results matching well with the experimental findings (Murugesh *et al*., 2013). From the simulated results it was inferred that the SA node cells failed to synchronize even upon variations in the free parameter pointing out the need for an external input, preferably a neural input (Murugesh *et al*., 2012; Murugesh *et al*., 2013). Hence an "integrate and fire" neuron model was developed that was injected as the preferred external input to the SA node cell. Also the authors wish to convey that the expansive studies carried out on the parameter (gap junction conductances) variations can be referred from (Murugesh *et al*., 2012, 2013) for adequate clarity.

Simulation studies

A Matlab code for the Sino Atrial node cell of a rabbit (RSA) was developed and its action potential (A.P) waveform was obtained by maintaining the gap junction conductance (parameter) of the cell at its nominal value. It was observed that the SA node cell oscillated with an intrinsic frequency of 266.67 bpm as in Figure 1.

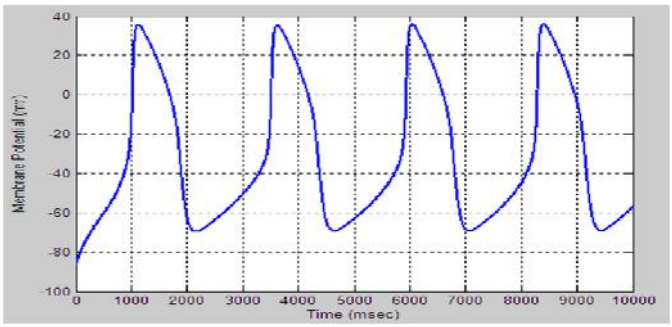

Figure 1. A.P of a Rabbit SA node cell for nominal parameter

Though the simulation was carried out for several minutes the time scale of all the action potential waveforms obtained have been changed accordingly to allow visibility and for ample clarity. Now the parameter value was effected a change of 20% decrease from its nominal value for which the SA cell oscillated with an intrinsic frequency of 253.33 bpm. For a variation of 10% decrease the cell oscillated with an intrinsic frequency of 266.67 bpm. Interestingly for the pattern of 20% and 10% increase in the parameter from its nominal value, the cell oscillated with the same intrinsic frequency of 280 bpm.

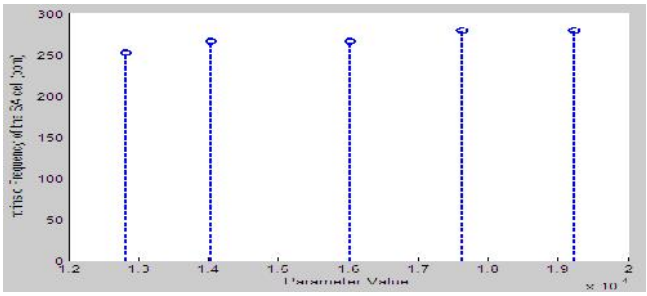

Figure 2. Parameter value vs Intrinsic Frequency

The plot of the variations obtained in the intrinsic frequency of the SA cell for different changes in the parameter value is seen in Figure 2. Once when the parameter value was exceeded in both ways it was observed that the SA node cell even failed to oscillate as inferred from Figure 3.

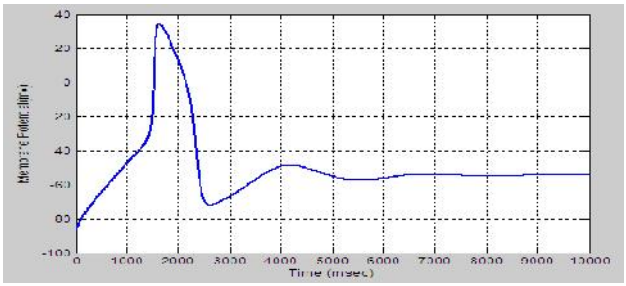

Figure 3. A.P of a RSA cell for excessive parameter variation

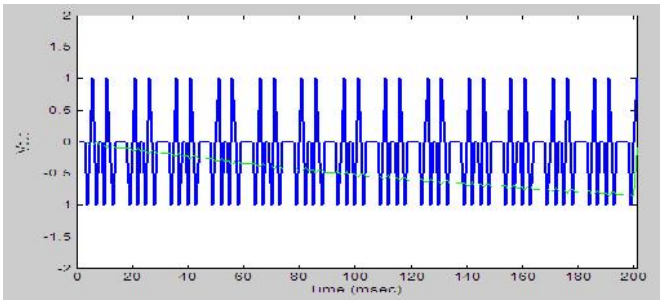

Figure 4. Scaled Integrate and Fire Neuron external input

The above results ascertained that for each and every value of the parameter setting the SA node cell can oscillate only at a finite rate, whereas beyond an extent the cell failed to oscillate at all. Also it was noted from Figure 2, by varying the parameter the SA node cell can be made to oscillate with an intrinsic frequency, from a minimum 253.33 bpm to a maximum of 280 bpm alone with no intermediate oscillatory rates possible. As proposed in the literature that the neural influence too can play a part in synchronizing the cardiac oscillators (Krishnan *et al*., 2005), an integrate and fire neuron model (IFN) emulating the role of the autonomic nervous system was developed. The same was injected as the much needed external input to the above single cell RSA model to enable it oscillate at varied intrinsic frequencies for differed gains. The so generated IFN model in which the time scale of the input pattern has been changed accordingly to allow visibility is shown in Figure 4. It was observed that the RSA cell responded with varied intrinsic frequencies for the different gain values of the applied external IFN input as evident from Figure 5. The IFN gain values that forced the SA node cell to oscillate at its least and utmost possible intrinsic frequencies by maintaining the parameter value was provided and the plot obtained is shown in Figure 5.

It was noted that for the highest gain value the intrinsic frequency of the cell was at its peak whilst the frequency went down for declining gain and their proportion was almost linear. The interdependence of the parameter value and the intrinsic frequency of the cell by maintaining the IFN gain value constant for each parameter setting were determined and presented in Figure 6. The above relationship in Figure 6 allows one to select a particular 'parameter value' to enable the cell exhibit oscillations at a predetermined intrinsic frequency for a maintained specific external gain input that can help in coaxing the SA node cell to beat in unison with an adjacent cell and thus possibly to regain synchronization. Now for the parameter maintained at its nominal value, the external gain input "k" was varied in both extremes. It was seen that the differed "k" when positive had the intrinsic frequency of the SA node cell at its peak value of 306.67 bpm whereas when negative the intrinsic frequency was as low as 240 bpm. Also intermediate intrinsic frequencies were obtained for a specific parameter itself unlike before (refer Figure 2). The plot of the same is given in Figure 7. By maintaining the nominal parameter a set positive gain value made the SA node cell to oscillate at its peak value of 306.67 bpm as compared to 266.67 bpm without an external input as seen in Figure 8.

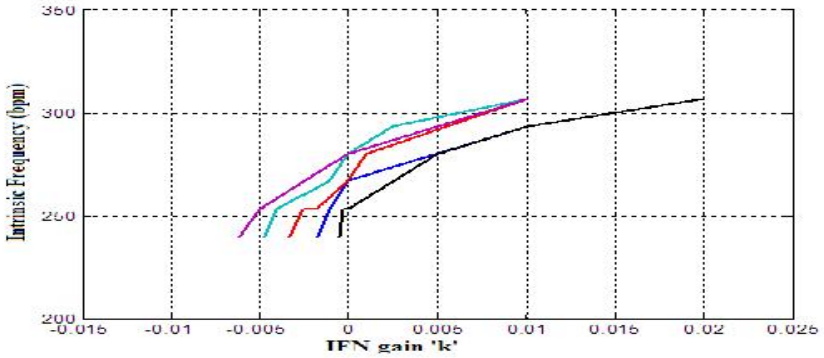

Figure 5. Intrinsic Frequencies of the RSA cell for differed gains

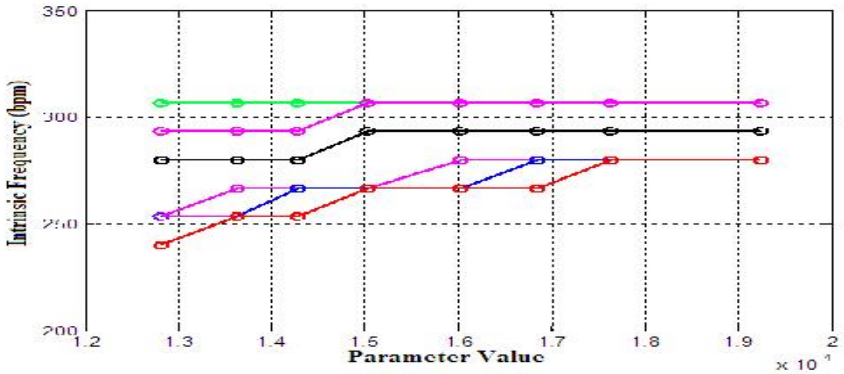

Figure 6. Interdependence of the Parameter and intrinsic frequency

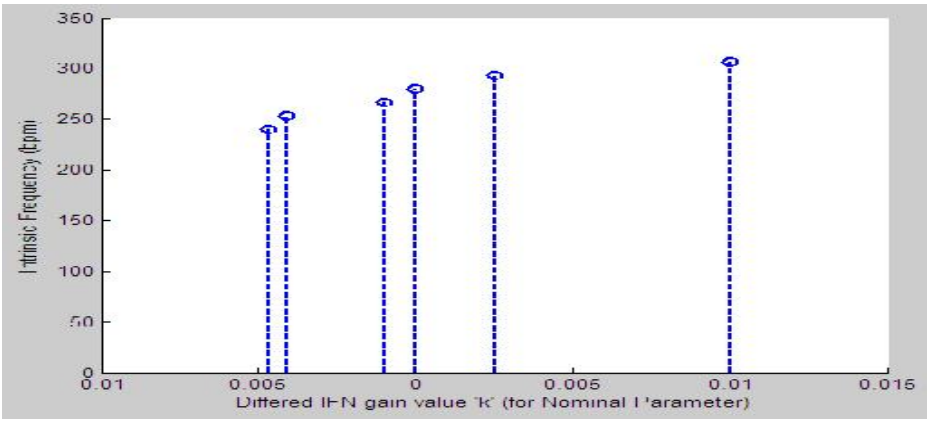

Figure 7. IFN Gain value vs Intrinsic Frequency (nominal parameter)

Now for a 20% decrease in the nominal parameter, a set +ve gain made the cell to oscillate at 306.67 bpm as compared to 253.33 bpm without an external input as seen in Figure 9. Similar work was carried out with 10% decrease from its nominal parameter for which a set positive gain made the cell to oscillate at an intrinsic frequency of 293.33 bpm as compared to 266.67 bpm without an external input as in Figure 10. Now a −ve IFN gain value was tried so as to decrease the intrinsic frequency of the SA node cell for 20% increase in its nominal parameter and the cell responded equally well. The set negative gain made the cell oscillate at 253.33 bpm as compared to 280 bpm without an external input as seen in Figure 11. Similar changes as before made the SA cell to reduce its oscillatory rate to 240 bpm as compared to 280 bpm without IFN for 10% increase in its nominal parameter as seen in Figure 12.

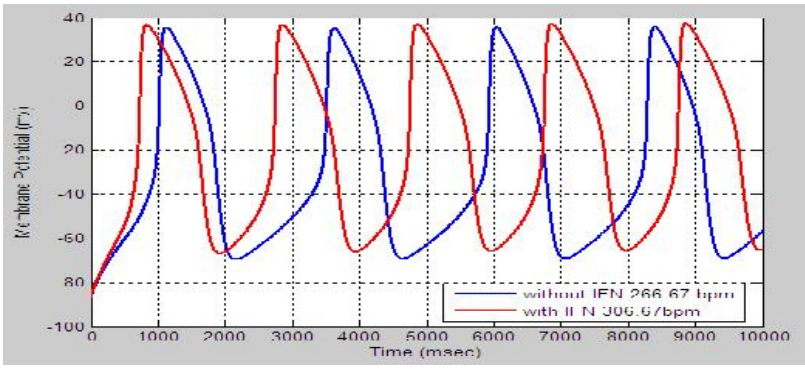

Figure 8. A.P of a RSA cell with +ve IFN gain for nominal parameter

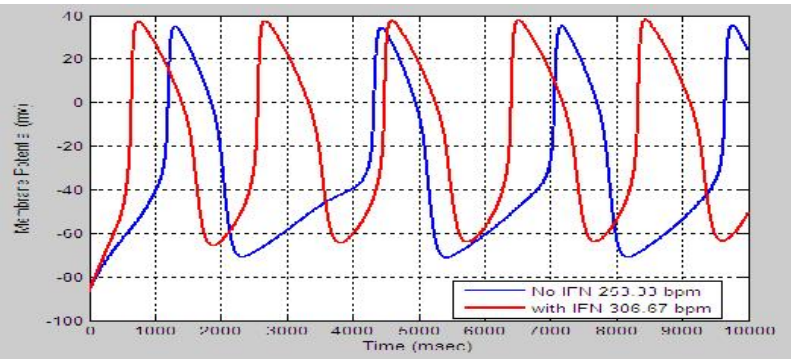

Figure 9. A.P with +ve IFN gain for 20% decrease in nominal parameter

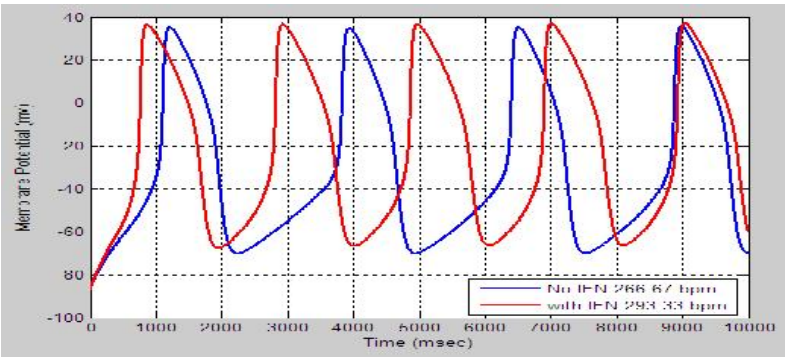

Figure 10. A.P with +ve IFN gain for 10% decrease in nominal Parameter

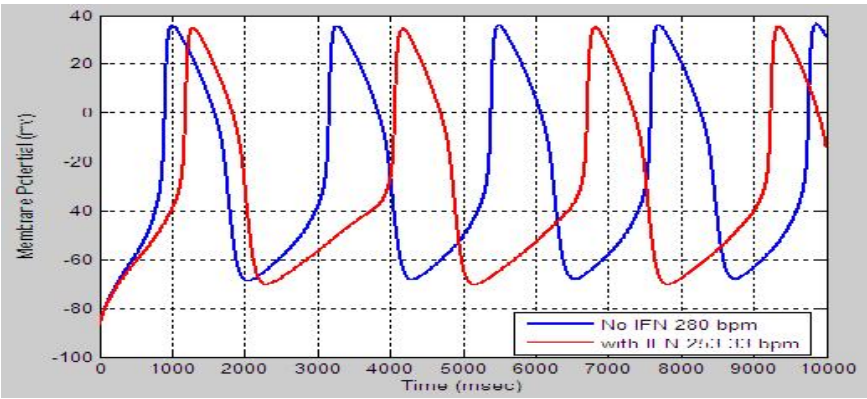

Figure 11. A.P with −ve IFN gain for 20% increase in nominal parameter

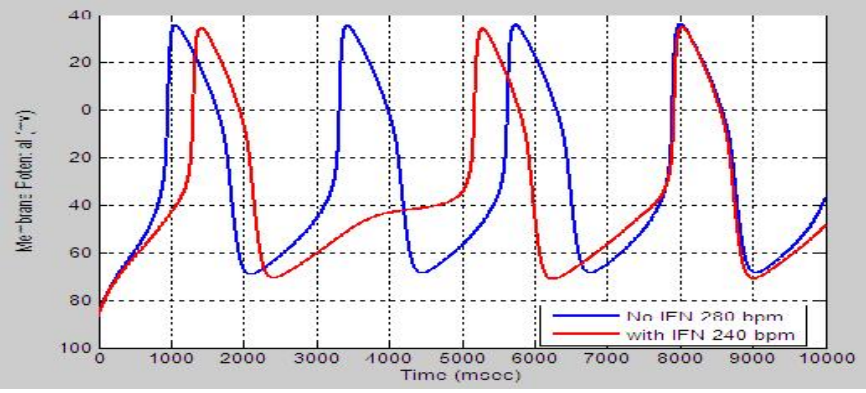

Figure 12. A.P with −ve IFN gain for 10% increase in nominal parameter

RESULTS AND DISCUSSION

From the simulation results it was observed that, though the parameter variations of a cardiac cell induced proportionate changes in its intrinsic frequency, it can coax the cells to oscillate only for a limited range of adjustment. Hence the extent to which the gap junctions alone can influence the cardiac cells to be in harmony is debatable. Keeping this in mind, an integrate and fire neuron model was developed and the applied external IFN input made the cells to exhibit oscillations with a varied intrinsic frequency that can be chosen by a predetermined gain value. Here for a finite parameter setting, the intrinsic frequency of the individual cell can be altered within the specified range by varying the IFN gain value in both extremes that can enhance the cell to be in unison with the adjacent cell. It is apparent that this becomes quite useful in order to step up/down the pace of a cardiac cell that is having a less/high intrinsic frequency to oscillate in accordance with adjoining SA cells, thereby aiding better synchrony. The simulation results quantify that the adjustable gain value of the externally applied IFN input can better aid the cells to undergo synchronization within the cardiac system.

REFERENCES

Bin He, Editor: Modeling and Imaging of Bioelectrical Activity, Principles and Applications, University of Minnesota Minneapolis, Minnesota Kluwer Academic/ Plenum Publishers, New York, Boston, Dordrecht, London, Moscow, 2004.

Bleeker WK, Mackaay AJC, Masson-Pevet M, Bouman LN, Becker AE, Functional and morphological organization of the rabbit sinus node. Circ Res 46: 11-22, 1980.

Bonke FIM, Electrotonic spread in the sinoatrial node of the rabbit heart. Pflugers Arch 339: 17-23, 1973.

Bruzzone R, White TW, and Paul DL, Connections with connexins: The molecular basis of direct intercellular signalling. *Eur. J. Biochem;* 238: 1-27, 1996.

Bukauskas FF, Veteikis RP, Gutman AM, Mutskus KS, Intercellular coupling in the sinus node of the rabbit heart. Biofizika22: 108-112, 1977.

Burkitt A N, A review of the integrate-and-fire neuron model: I. Homogeneous synaptic input, Biol Cybern 95: 1–19, 2006.

Burkitt A N, A review of the integrate-and-fire neuron model: II. Inhomogeneous synaptic input and network properties, Biological Cybernetics, issue 2, pp 97-112, August 2006.

Chadwick D J and Goode J, Editors: Development of the Cardiac Conduction System, Novartis Foundation Symposium 250. Volume 250, Novartis Foundation, John Wiley & Sons, 2005.

DeHaan RL, In vitro models of entrainment of cardiac cells. In Cardiac Rate and Rhythm, edited by LN Bouman, HJ Jongsma. The Hague, Martinus Nijhoff, pp 323-361, 1982.

Delmar M, Jalife J, Michaels DC, Effects of changes in excitability and intercellular coupling on synchronization in the rabbit sino-atrial node. *J Physiol (Lond)* 370: 127-150, 1986.

Demir S S, J.W. Clark, C.R. Murphey, and W.R. Giles, "Modeling in Physiology, mathematical model of a rabbit sinoatrial node cell," The American Physiological Society, 0193-1849/94, 1994.

Denyer JC and Brown HF, "Rabbit sinoatrial node cells: isolation and electrophysiological properties," *J Physiol (Lond)* 428:405-424, 1990.

Dhein S, "Cardiac Gap Junctions-Physiology, Regulation, Pathophysiology and Pharmacology," S Karger AG, P.O. Box, CH–4009 Basel (Switzerland) 1998.

DiFrancesco D and Noble D, A model of cardiac electrical activity incorporating ionic pumps and concentration changes, Phil. Trans. R. Soc. Lond. B 307, 353-398, 1985.

Gerstein GL, Mandelbrot B, Random walk models for the spike activity of a single neuron. *Biophys J* 4:41–68, 1964.

Gerstner W and Kistler WM, Spiking neuron models: single neurons, populations, plasticity. Cambridge University Press Cambridge, 2002.

Goodenough D A and Paul D L, "Gap Junctions," Cold Spring Harb Perspect Biol, 2009.

Goshima K, Synchronized beating of and electronic transmission between myo cardial cells mediated by heterotypic strain cells in monolayer culture, Exp. Cell. Res. 58, 420-426, 1969.

Guyton A C and Hall J E, "Textbook of Medical Physiology", Eleventh Edition, Elsevier, 2007.

Harary I and Farley B, In vitro studies in single beating rat heart cells: II. Intracellular communication, Exp. Cell. Res. 29: 466-474, 1963.

Hausser M, The Hodgkin-Huxley theory of the action potential, Nature: Neuroscience supplement, page 1165, volume 3, November 2000.

Hilgemann, D., and Noble D. Excitation-contraction coupling and extracellular calcium transients in rabbit atrium: reconstruction of basic cellular mechanisms. Philos. Trans. R. Soc. Lond. B Biol. Sci. 230: 163-205, 1987.

Ikeda Noriaki, Model of bidirectional interaction myocardial pacemakers based on the phase response curve, Biol. Cybern. 43 157-167, 1982.

Jalife J, Mutual entrainment and electrical coupling as mechanisms for synchronous firing of rabbit sino-atrial pacemaker cells. J Physiol (Lond) 356: 221-243, 1984.

Joyner R W and Van Capelle F J L, Propagation Through Electrically Coupled Cells, "How a Small SA Node Drives a Large Atrium", Biophys. J. Volume 50, 1157-1164, December 1986.

Kandel ER, Schwartz JH, Jessell TM, Principles of Neural Science, 4th edition. McGraw-Hill, New York 2000.

Kovacic G, Models of Neuronal Dynamics, http:// www.rpi. edu/dept/math/ms_undergraduate/PDF/Kovacic.pdf, 2001.

Krishnan J, Chakravathy V S, Radhakrishnan S, Nayak V, Solomon Victor, "Neural Influence is essential for synchronizing cardiac oscillators: A computational model, "IJTCVS,21,262-268,2005.

McAllister RE, Noble D & Tsien RW, Reconstruction of the electrical activity of cardiac Purkinje fibres. *J Physiol* 251, 1–59, 1975.

Mel B.W, Synaptic integration in an excitable dendritic tree. *J. Neurophysiol.* 70, 1086–1101, 1993.

Murugesh T S, Krishnan J and Malathi R, Certain investigations on the gap junction conductance in synchronization issues, *IJRRAS* Volume 15 Issue 3, pp 312-321, June 2013.

Murugesh T S, Krishnan J and Malathi R, Effects of Gap Junction Conductance in Synchronizing Cardiac Oscillators, CIIT International Journal of Biometrics and Bioinformatics, Volume 4, No 10, pp 454-458, September 2012.

Noble D, A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pacemaker potentials. *J. Physiol.,* Vol. 160, p. 317–352, 1962.

Noble D, Alan Garny and Penelope J. Noble, How the Hodgkin–Huxley equations inspired the Cardiac Physiome Project, *J Physiol* 590.11 pp 2613–2628, 2012.

Noble D, From the Hodgkin-Huxley axon to the virtual heart, *J Physiol* 580.1 pp 15-22, 2007.

Opie LH: The Heart Physiology and Metabolism. New York, Raven Press, 1991.

Poirazi P, Brannon T and Mel, B.W, Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. Neuron 37, 977–987, 2003a.

Poirazi P, Brannon, T. and Mel, B. W, Pyramidal neuron as 2-layer neural network. Neuron 37, 989–999, 2003b.

Pratt O, Carl Gwinnut and Sarah Bakewell, The Autonomic Nervous System - Basic Anatomy and Physiology. Update in anaesthesia, Compiled from articles originally published in Anaesthesia Tutorial of the Week and Update in Anaesthesia, edition 5 (1995) pp 36-39, 2005.

Sano T, T. Sawanobori, H.Adaniya, Mechanism of rhythm determination among pacemaker cells of the mammalian sinus node, *Am. J. Physiol.* 235 H379-H384, 1978.

Schwarzmann G, Weigandt H, Rose B, Zimmermann A, Ben Haim D, Loewenstein WR: Diameter of the cell-to cell junctional membrane channels as probed with neutral molecules. Science; 213: 551–553, 1981.

Simpson I, Rose B, Loewenstein WR: Size limit of molecules permeating the junctional membrane Channels. Science; 195: 294–296, 1977.

Sperelakis N: Propagation mechanisms in the heart. Annu Rev Physiol ; 41: 441–457, 1979.

Strogatz SH. "Nonlinear Dynamics and Chaos: With Applications in Physics, Biology, Chemistry, and Engineering (Studies in Nonlinearity)," Addison Wesley, 1994.

Trautwein W and Uchizono K. "Electron microscopic and electrophysiologic study of the pacemaker in the sinoatrial node of the rabbit heart," Z Zellforsch;61:96–109, 1963.

Weingart R and Maurer P: Action potential transfer in cell pairs isolated from adult rat and guinea pig ventricles. Circ Res; 63: 72–80, 1988.

West A and Belardinelli L, Sinus slowing and pacemaker shift caused by adenosine in rabbit SA node. Pflugers Arch 403**:** 66-74, 1985.

Winfree AT, The Geometry of Biological Time. Interdisciplinary Applied Mathematics; v.12, second edition, New York, Springer-Verlag, 2001.

Yang Qu and Dahl G, Function of the voltage gate of gap junction channels: Selective exclusion of molecules, PNAS,vol. 99, No. 2, 697–702, 2002.

****