The Molecular Biological Application of the Theory of Stochastic Resonance: The Cellular Response to the ELF AC Magnetic Field

Hsien-Chiao Teng

Department of Electrical Engineering, Chinese Military Academy, Fengshan, Taiwan 833, Republic of China, scteng@cc.cma.edu.tw; 011886-7747-9510 ext 134

Abstract: The cultured rat liver epithelial cells in vitro initiates noisy magnetic fluctuation, which can be measured and provides a basis for signal amplification to transmit of weak signal along the possible signaling pathways in cell despite low signal-to-noise ratio of the primary cellular response for external stimulus. By using power density spectrum analysis of noisy magnetic fluctuation, the signal-to-noise ratio (SNR) of the possible intrinsic periodical extremely low frequency signals can be depicted. The calculation reveals that 7 Hz is an intrinsic signal for rat liver epithelial cell system. Exposing cultured rat liver epithelial cells into a constant extremely low frequency (ELF) alternating current (AC) magnetic field 150 mG at 7 Hz for 60 minutes, 20% promotion of the gap junction intracellular communication (GJIC) was observed from Lucifer yellow fluorescence microscopic image while comparing with the control. Cellular response is experimentally found at 7 Hz, which agrees with the mathematical analysis under the theory of stochastic resonance. [Nature and Science. 2005;3(1):37-41].

Key words: signal to noise ratio; gap junctional intracellular communication (GJIC); power density spectrum; stochastic resonance

1 Introduction

The theoretical calculation of a periodically driven stochastic process has been developed for two decades and applied to a wide variety of naturally occurring and computer simulation processes (McNamara and Wiesenfeld, 1989; Jung, 1993; Jung et al., 2005; Schatzer and Weigert, 1998; Schmid et al., 2004). The possibility of amplification of weak

signal in cellular system and the modulation of grating properties of membrane K+ and Na+ channels by external signals were proposed under the assumption of existence of stochastic resonance (Schmid et al., 2004). However, the gap junctional intracellular communication (GJIC) within the cells may induce the surface-current instead of trans-membrane voltage upon the cultured cells (Hart, 1996). In cell, six connexin 43 subunits oligomerze in the Golgi apparatus into a connexon, called hemi channel and be transported to plasma membrane of the cell. Before pairing process, hemi channels are closed to avoid leakage of cellular contents and entry of extra-cellular materials. During the pairing of connexons and aggregation into plaques at the plasma membrane, connexin 43 is phosphorylated at least twice and connexons are attracted to those located on the adjacent cells. Two connexons join in an end-to-end manner to form a complete channel. The channels aggregate into large gap junction plaques open to connect two cells for cell-to-cell communication and is called gap junctional intracellular communication (GJIC), which can be modulated by environmental factors, such as drugs, X-ray, electromagnetic fields etc. Since the function of the GJIC, cultured cells coupled together in vitro except the stem cells and cancer cells (Trosko et al., 1990). In this article, we introduce the magnetic field fluctuation induced by GJIC surface-current of the cells. We apply the concept of periodically driven stochastic processes to a model consisting of hemi channels and Lucifer yellow fluorescence diffusion. Scrape loading dye transfer of Lucifer yellow is a technique to observe and measure the diffusive range of Lucifer yellow fluorescence (Upham et al., 1998). The varied diffuse range of Lucifer yellow fluorescence can express the cellular response under the exposure of ELF at the intrinsic-resonance frequency ω. Since GJIC is affiliated with many pathological endpoints (Trosko et al., 1990; Upham et al., 1998; Trosko et al. 2001), GJIC modulation can be used as a biological response factor to evaluate the ELF reaction for cellular system.

2 Theory

Electronically, the magnetic fluctuation can be acquisitioned to oscilloscope voltage V(N) for N times measurement per second.

$$V(N)=\{V_1,V_2,\ldots..V_{N-1}V_N\}; \quad (1)$$

$$R_q=\left(\frac{1}{N}\right)\sum_{p=1}^{N}V_pV_{p+q} \quad (2)$$

$$S_k = \sum_{q=1}^{N} R_q e^{\frac{i2\pi}{N}kq} \quad (3)$$

$i = \sqrt{-1}$. Equation (3) is the power density component at frequency $\omega_k = \frac{2\pi}{N}$ k (fundamental frequencies), the unit of S_k is watt per hertz for each V_p. To assess the magnitude of amplification of a signal, we take the surface diffusive current fluctuation of the cultured cells through N measurements per second. Begin from a simple open-close gap junctional connexin 43 channel assumption, whose close state and open state specified by c-state and o-state respectively, the rate of changing of c-state and o-state affects the surface diffusive current fluctuations across the cells. However, we must propose the state of the channel be either fully open or close. The probabilities P^o and P^c represent the states, which is either in c-state or in o-state respectively (Galvanovskis et al., 1997). Then, the diffusive current equation for connexin 43 channels can be written as

$$<I> = \sum_{k=1}^{m} kP(k) \quad (4)$$

where probability P(k) indicates total m channels is taken into account for opening k channels from all cell-to-cell communications on the surface of the cell mono layer. Therefore,

$$P(k) = \frac{m!}{k!(m-k)!}(P^o)^k (P^c)^{m-k} \quad (5)$$

$$\frac{dP^o}{dt} = r^c P^c - r^o P^o \quad (6)$$

$$\frac{dP^c}{dt} = r^o P^o - r^c P^c \quad (7)$$

where r^c is the rate of changing from c-state to o-state and r^o is the rate of changing from o-state to c-state of the connexin 43 channels activating totally on the cells mono layer surface. Generally, r^o does not have to be same with r^c since the life times of the o-state and c-state may vary. To clarify the physical meaning, we further assume the current through an open channel as i. The diffusive current caused by GJIC channels can be rewritten as

$$<I> = m\, i\, P_S^o \quad (8)$$

where P_S^o is the modulated probability for o-state by external ELF field signal. According to theory of Jung (1993), the power spectral component originated from the signal is given by

$$S_k = \frac{(m\,i)^2}{2} \sum_{q=1}^{\infty} \left| C_q \right| \delta(\omega - q\omega_k) \quad (9)$$

C_q is the Fourier expansion coefficients of P_S^o.

In Comparison with equations (3) and (9), the signal-to-noise ratio (SNR) of the characteristic frequency of the cell system can be depicted (Galvanovskis et al., 1997).

$$SNR = \left| \frac{\text{signal amplitude}}{\text{background amplitude}} \right|^2 = A^2 \left| \sqrt{m \frac{\pi}{\Delta\omega} \frac{r^o r^c}{(r^o + r^c)}} \right|^2 \quad (10)$$

where m is the number of channels, A is the amplitude and $\Delta\omega$ is the bandwidth of the external ELF field signal.

3 Materials and Methods

Fitting 3.5 cm diameter cell culture dish, a solenoid was made by a simple 5-cm diameter plastic cylinder tube 2 cm in height wrapped with single layer 200 turns 0.45-mm diameter cooper string connecting a function generator to provide ELF field signals for rat liver epithelial cells *in vitro*. The solenoid was placed in an incubator so called ELF incubator controlled the environment at 5% CO_2 at 98% relative humidity. Another sham field chamber was exactly same as the ELF incubator only with no ELF provider. The cell culture dishes were placed within the solenoid parallel to the normal direction of the cross-section. The function generator generated the ELF signal through the solenoid perpendicularly to the cells in the center of the solenoid for sixty minutes.

3.1 Cell Culture

The rat liver epithelial cell line *in vitro* was obtained from the Fisher Scientific (WB344) (Hampton, NH, USA). It was derived from normal liver and maintained in D-medium (Formula 78-5470EF, GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (GIBCO) and 50 μg/ml gentamicin (Quality Biological, Inc., Gaithersburg MD, USA). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air and were fed or trypsinized every two to three days.

3.2 SNR Spectrum

By using of the probe of Gauss-meter, we took rat liver epithelial cells-induced magnetic fluctuation $\{B_i^c\} = \{B_1^c, B_2^c \ldots\ldots B_{2000}^c\}$, which may contain the

cellular response signal to the external ELF magnetic field reaction. The sampling time was 0.0005 second and the probe was at the distance 10^{-4} m perpendicularly to the center of single layer of the cells. The Gauss-meter was manufactured by F.W. Bell Company (series of 9550) in Florida of USA. Oscilloscope was manufactured by Agilent Company (54621A) and be able to convert $\{B_i^c\}$ to voltage sequence $\{V_i^c\}$. Matlab and Fortran programming were used for power density spectrum analysis of these voltage sequences. Medium control group $\{B_i^m\}$ was also taken at 2000 times per second at the distance 10^{-4} m perpendicularly to the bottom of culture plate only with medium in it. In the mean time, geo-field control group $\{B_i^n\}$ was taken with the same sample rate at the distance 10^{-4} m perpendicularly to the bottom of empty culture plate for recording local geomagnetic field fluctuation. The $\{V_i^m\}$ and $\{V_i^n\}$ voltage sequences were recorded in the same way as $\{V_i^c\}$ did previously. Further more, we must take a series trial signals for separating the characteristic frequencies from the background. Those signals were defined as $\Omega_i(n)=A_i\times\sin(\omega_i n)$, $1\text{Hz} \le \omega_i \le 60\text{Hz}$, where $A_i = F\times V_{.,\max}^{i}$, F is the adjustable fraction factor and $V_{.,\max}^{i}$ is such, for instance, $V_{.,\max}^{i}$ = MAX($\{V_i^c\}$), as to the maximum value of the sequence. By taking into consideration of signal amplitudes $A_{0.7} = 0.7\times V_{max}$, $A_{0.4} = 0.4\times V_{max}$, $A_{0.03} = 0.03\times V_{max}$ for a given trial signal at ELF ω_i ($1\text{Hz} \le \omega_i \le 60\text{Hz}$), we computed the Fourier transforms of the autocorrelation function of $\{V_i^c + \Omega_i(n)\}$ to obtain the signal-to-ratio ratio $SNR\omega_i$ (0.7), $SNR\omega_i$ (0.4) and $SNR\omega_i$ (0.03) respectively. The SNR spectrum for $\{V_i^c + \Omega_i(n)\}$ at frequency ω_i could be simply a second order equation as $a \times (SNR\omega_i(F))^2 + b \times (SNR\omega_i(F)) + c = 0$. Accordingly, substituting the SNR values into the equation, we can solve unknowns a, b and c

$a(SNR\omega_i(0.7))^2 + b(SNR\omega_i(0.7)) + c = 0$; (11)

$a(SNR\omega_i(0.4))^2 + b(SNR\omega_i(0.4)) + c = 0$; (12)

$a(SNR\omega_i(0.03))^2 + b(SNR\omega_i(0.03)) + c = 0$ (13)

Equations (11), (12), (13) involved three equations and three unknowns, a, b and c. Therefore, a, b and c values could be solved. If c-value is bigger than zero, which means the SNR of the intrinsic signal peaked at ELF ω_i is detected. In the paper by Galvanovskis (1997), $SNR\omega_i(F) = (F\times V_{max})^2 \times m \times Q \times \frac{2\pi}{\omega_i(\tau_o + \tau_c)}$, the theoretical value of c is approximately 0.09 when the life time of c-state and o-state equal to 10^{-6} second. Under optimal condition, the quality factor $Q = \frac{\omega_S}{\Delta\omega}$ approximately equals to 100 at 60Hz with bandwidth $\triangle\omega = 0.6$Hz, F = 0.6 respectively. The numbers of GJIC channels are approximately taken 1000 per cell (Galvanovskis et al., 1997).

3.3 Bioassay of GJIC

The scrape load/dye transfer (SL/DT) technique was used to measure the GJIC within cells. After exposure to ELF at intrinsic frequency, the cells were rinsed with phosphate buffered saline (PBS), and a PBS solution containing 4% concentration Lucifer yellow fluorescence dye is injected into the cells by a scrape using a scalpel blade. Afterwards the cells were incubated for 3 min and extra cellular dye was rinsed off and fixed with 5% formalin. We then measured the area of the dye migrated from the scrape line using digital images taken by an epifluorescent microscope and quantitated with Nucleotech image analysis software (Upham et al., 1998) for the GJIC images.

4 Results

Figure 1 depicts the plot of V_i^c. Figure 2 depicted the SNR fitting curve of ELF at 7Hz such as to confirm if the intrinsic frequency situated. When the intrinsic ELF is present at 7 Hz, GJIC modulation can then be used to observe the biological effect of ELF provided externally. Figure 3 shows the GJIC fluorescent images. Since the GJIC of cells was quantified with the measurement of the average distance of dye migration, GJIC was reported in this article as a fraction of the control (FOC) in Figure 4. An FOC value equals to 1.0 indicates normal GJIC. The FOC value more than 1.0 indicates excitation.

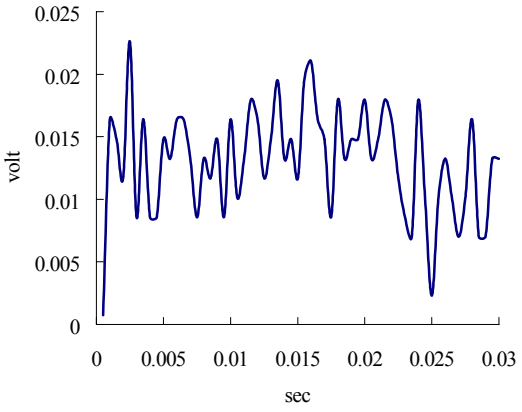

Figure 1. V_i^c schematic drawing

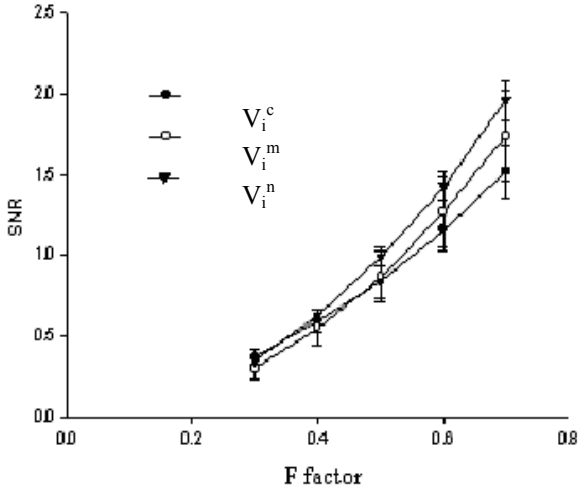

Figure 2. SNR of the trial signal at 7 Hz

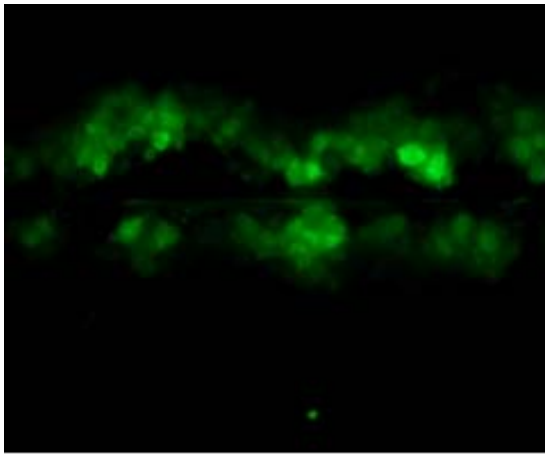

Figure 3. The GJIC imagine

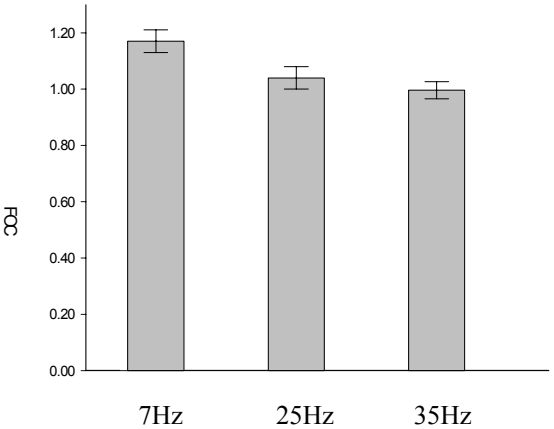

Figure 4. The fraction of control of the GJIC Assay at different ELF exposure

5 Discussion

Experimental results depicted that the GJIC within cells relates to both the background noisy magnetic field fluctuation and the intrinsic ELF signal. In the paper by Hart, the GJIC affects the cell-surface current, which is about 10^{-9} Amp (Hart, 1996) under the background noisy magnetic fluctuation within confluent cells in culture. Thus, the GJIC would also affects cell surface electrical current simultaneously when the SNR of the intrinsic signal adjusts the changing rate of the GJIC channel in o-state. The varying amplitudes of trial signal were used to determine the intrinsic signal and its SNR buried in the power density spectrum of $V_i^c(t)$.

6 Conclusion

The main feature of our research introduced is $V_i^c(t)$ relating to the change in the probability of GJIC channels being in o-state or c-states. The $V_i^c(t)$ expression for cell induced GJIC current flow into and out of the two states, o-state and c-state *in vitro* under the background magnetic fluctuation has been identified by specific external ELF ac magnetic field signal at 7 Hz, which modulates the GJIC 20% within the cells. Based on the application of stochastic resonance, which predicts the existence of the intrinsic ELF signal, our study depicted that we were able to obtain the corresponding level of the SNR expression for illustrating that the external ELF ac magnetic field signal can modulate 20% GJIC promotion within the cells at 7 hertz.

Correspondence to:
Hsien-Chiao Teng
Department of Electrical Engineering
Chinese Military Academy, Fengshan, Taiwan 833
Republic of China
Email: scteng@cc.cma.edu.tw
Telephone: 011886-7747-9510 ext 134

References

[1] Hart F. Cell culture dosimetry for low frequency magnetic fields. Bioelectromagnetics 1996;17:48-57.
[2] Galvanovskis J, Sandblom J. Amplification of electromagnetic signals by ion channels. Biophysical Journal 1997;73:3056-65.
[3] Jung P. Periodically driven stochastic systems. editor: I. Procaccia, Physics Reports (Review Section of Physics Letters) 1993;234(4,5);175-295.
[4] Jung J., Alexander N, Muhammad KN, Afghan N, Suhita, Ghanim U. New Journal of Physics *2005;*7:17 (http://www.njp.org/)
[5] McNamara B, Wiesenfeld K. Theory of stochastic resonance. Physical Review A 1989;39(9):4854-69.
[6] Schatzer L, Weigert S. Solvable three-state model of a driven double-well potential and coherent destruction of tunneling. Physical Review A 1998;57(1):68-78.
[7] Schmid G, Guychok I, Hanggi P, Zeng S, Jung P. Stochastic resonance and optimal clustering for assemblies of ion channels.

Fluctuation and Noise Letters 2004;4(1):L33-L42.
[8] Trosko JE, Chang CC, Madhukar BV. Modulation of intercellular communication during radiation and chemical carcinogenesis. Radiation Research 1990;123:241-51.
[9] Trosko JE, Chang CC. Role of stem cells and gap junctional intercellular communication in human carcinogensis. Radiation Research 2001;155:175-80.
[10] Upham BL, Deocampo ND, Wurl B, Trosko JE. Inhibition of gap junctional intracellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. Int J Cancer 1998;78:491-5.