Mouse Osteoblast Cell Sensitivity to the AC Magnetic Field at 14 Hz

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Abstract: Since the surface electrical current of the attached mouse osteoblast cell monolayer in a culture plate could induce magnetic fluctuation, power density spectrum of the geomagnetic fluctuation (GF) recorded at the sample rate 2000 times per second illustrated the signal to noise ratio (SNR) spectrum in a frequency interval (0 ~ 60 Hz) for the mouse osteoblast cells system. In contrary to the background, 14±2 Hz was an intrinsic signal within cells. We exposed cells into a constant extremely low frequency (ELF) magnetic field (~ 50 m Gauss) at 14 Hz for 60 minutes, 20% modulation of the gap junction intracellular communication (GJIC) within mouse osteoblast cells was observed from the analysis of Lucifer yellow fluorescence microscopic image while comparing with the control group. Cellular response, GJIC modulation caused by the ELF magnetic field applied through ac function generator and solenoid coil was relied on the resonance within cells at intrinsic frequency. [Nature and Science 2003;1(1):27-31].

Key words: power sensitive spectrum, signal to noise ratio, GJIC

Introduction

The attention of the reaction of ELF magnetic field for biological system has been discussed a lot. Researches have focused on two major concerns for twenty years. The first one is public concern about hazard and health effect. The other one is the scientific interest, which goes further to answer if biological system reacts to ELF magnetic field; even ELF provides less energy than thermal motion (Adair, 1991; 1992; 1994). However, no clinical evidence has shown any human health effect by ELF magnetic field and no mechanism can clearly explain every observed biological effect for the biological system under ELF magnetic field (Takebe, 1999; Adair, 1994; EPRI, 1994). In addition, some experimental observations reported in a laboratory cannot be duplicated in another laboratory. The confliction of the unduplicated experimental observations makes the study of biological effect under ELF magnetic field more difficult. Nevertheless, if ELF magnetic field influences the cell membrane surface electrical current, the form of the reaction of the biological system under ELF magnetic field can be electrical signals. Eugene recognizes deterministic, stochastic, fractal and chaotic signals (Eugene, 2001) as four different type biological signals. A deterministic signal is one whose values in the future can be predicted if enough information about its past is known. Stochastic signals are signals for which it is impossible to predict an exact future value even if one knows it's entire past history. Fractal signals have the property that they look very similar at all levels of magnification, which is referred as scale-invariance. Chaotic signals are deterministic signals with sensitive dependence on some conditions that cannot be predicted exactly in the future. Therefore, we assume the signals from cells induced membrane surface electrical current can affect geomagnetic fluctuation B(t). In addition, we can measure B(t) by using the probe of gauss-meter, transform it to oscilloscope voltage as V(t) and digitally record it at the rate of 2000 times in a second. Mathematically, we write it as follows:

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

Take the autocorrelation coefficients of V(t)

$$R_q = \left(\frac{1}{N}\right)\sum_{p=1}^{N} V_p V_{p+q}$$

Perform the Fourier expansion of R_q

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

S_k is the component at frequency k and the unit of is watt per frequency.

$\omega_k = \frac{2\pi}{N}$ k (fundamental frequencies) for V_p ; $i = \sqrt{-1}$.

If N~ ∞,

$$S(\omega) = \int_{-\infty}^{\div\infty} R(\tau) e^{-i\omega\tau} d\tau \qquad (1)$$

$$R(\tau) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} S(\omega) e^{-i\omega\tau} d\omega \qquad (2)$$

$R(\tau) = \langle V(t+\tau)V(t) \rangle$, τ is the time interval and S(ω) is the average power for V(t). Equations (1) and (2) are the Fourier transform pairs and V(t) is a stationary process which means changing the original point of V(t) will not affect the statistical characteristics. Contrarily, $\int_{-\infty}^{+\infty} S(\omega)d\omega$ is the total energy of the system, the power of the signal can be calculated from power density spectrum and the SNR of the intrinsic signal can be calculated. In this report, we take N=2000 and calculate the SNR spectrum of the mouse osteoblast cells system from 5 to 60 Hz. Relying on surface electrical current distribution induced within cells, ELF magnetic field may cause gap junctional intracellular communication (GJIC) modulation (Hart, 1996). Since GJIC is affiliated with many pathological endpoints (Trosko, 1990, 2001; Upham, 1998), we use GJIC as a factor to evaluate the ELF magnetic field reaction for mouse osteoblast cell system. Scrape loading dye transfer of Lucifer yellow is used to measure gap junction intracellular communication (GJIC) modulation under the exposure of ELF magnetic field. The intrinsic resonance detected in SNR spectrum of the mouse osteoblast cells system is very likely to be a chaotic signal, which is not fully predictable.

Materials and Methods

For the diameter of the culture dish used was 3.5 cm, we used a simple 5-cm radius helical coil, which is wrapped with 200 turns 0.45-mm diameter cooper string around a plastic cylinder tube connected to the function generator for input ELF signals. The whole system was placed in an incubator. The 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 exposure to the ELF. The cell culture dishes were placed perpendicular to the input ELF. The function generator generated the ELF signal through the solenoid applied to the cells located in the center of the solenoid for sixty minutes.

Cell Culture

Mouse osteoblastic MC3T3-E1 cell line was obtained from: D.T. Yamaguchi, Research Service and Geriatrics Research, Education, and Clinical Center, VAMC, West Los Angeles, California, USA. It was cultured in D-medium (Formula 78-5470EF, GIBCO, Grand Island, NY), supplemented with 5% fetal bovine serum (GIBCO) and 50 µg/ml gentamicin. The cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2 and 95% air and were fed or split every two to three days. Upon confluency, the osteoblastic cells could be induced to differentiate by the addition of 50 g/ml ascorbic acid alone or ascorbic acid plus 7 mM ß-glycerophosphate (ß-GP) to the medium for 1 month.

Detection SNR of Intrinsic

We used a probe of Gauss-meter to measure the $B_{cigf}(t)$, which is the coupled fluctuation of geomagnetic field and cells induced magnetic fields. The probe was located vertically 10^{-4} meter to the mono-layer of the cells attached at the culture dish. The Gauss-meter was manufactured by F.W. Bell Company (series of 9550) in Florida, USA. Oscilloscope transformed $B_{cigf}(t)$ to electrical voltages as $V_{cigf}(t)$. The oscilloscope was manufactured by Agilent Company (54621A). By using the software also provided by Agilent Company, we collected the digital $V_{cigf}(t)$ from the oscilloscope. In contrast, $V_{gf}(t)$ and $V_{migf}(t)$ were also measured. V_{gf} was only earth magnetic fluctuation and m V_{migf} was the magnetic fluctuation of the medium in culture plate without cells. Matlab and Fortran computer languages were used for power density spectrum analysis. The sample rate was taken 2000 times in a second. The following steps identified intrinsic frequency and its SNR:

Let ω = {1 Hz, 2 Hz, …20 Hz, 21 Hz, 22 Hz……58 Hz, 59 Hz} denote the set of frequency ω_i of the given test signal. Sine(ω_it), simulated by Matlab software, from 1 Hz to 59 Hz, where ω_1 = 1 Hz, ω_2 = 2 Hz, ω_3 = 3 Hz,….. ω_{50} = 50 Hz, …ω_{59} = 59 Hz, respectively.

1. Take the maximum V_{max} from $V_{gf}(t)$
2. Add sine(ω_it) with amplitude V_{max} to $V_{gf}(t)$ and name the result as $V_{gftest}(t)$.
3. Perform autocorrelation and Fourier transform to get the power density spectrum (PDS) of $V_{gftest}(t)$.
4. Calculate the SNR, which can be represented as $S_{max}(\omega_i)$ for each ω_i from PDS of $V_{gftest}(t)$ to get SNR spectrum. Be noted that (a) the power of the signal is defined as the area under the frequency at ω_i, and (b) the power of the noise is defined as the total power of the $V_{gf}(t)$, and (c) the SNR is defined as the power of the signal divided by the power of the noise.
5. Repeat step 2, step 3 and step 4 with the amplitudes 0.7 V_{max}, 0.4 V_{max}, 0.03 V_{max}, respectively for the given test signal at every frequency ω_i to get $S_{0.7}(\omega_i)$, $S_{0.4}(\omega_i)$ and $S_{0.03}(\omega_i)$.
6. Assume $pX^2 + qX + r = 0$, using square curve for fitting data,
   $pS_{0.7}(\omega_i)^2 + q\ S_{0.7}(\omega_i) + r = 0$
   $pS_{0.4}(\omega_i)^2 + q\ S_{0.4}(\omega_i) + r = 0$
   $pS_{0.03}(\omega_i)^2 + q\ S_{0.03}(\omega_i) + r = 0$

Since there are three unknowns p, q and r, with three known equations, we can easily calculate the value of r,

which is the SNR of the intrinsic frequency ω_i at the amplitude of test signal ω_i equals to zero. After we found the intrinsic frequency, we expose cells at intrinsic frequency provided by function generator in the center of solenoid. We used the same steps to calculate the SNR of intrinsic for the observations of $V_{cigf}(t)$ and $V_{migf}(t)$.

Bioassay of GJIC

The scrape load/dye transfer (SL/DT) technique was used to measure GJIC. After exposure to either sham or ELF, the cells were rinsed with phosphate buffered saline (PBS), and a PBS solution containing a small molecular weight fluorescent dye, 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, 1998) for the GJIC images.

Results

Figure 1 is the figure of V_{cigf}. Figure 2 has shown the correlation between the SNR and the amplitude of the test signal while fitting curves for V_{cigf}, V_{migf} and V_{gf}. The intercept of the curve was at 0.005, which is the intrinsic SNR value for the test signal at 14 Hz. Therefore, if 14 Hz modulates GJIC, GJIC modulation can work as an index to find the biological effect at intrinsic ELF. We demonstrated the GJIC fluorescent image in Figure 3 and Figure 4. Since the GJIC in cells was quantified with the measurement of the average distance of dye migration, GJIC was reported as a fraction of the control (FOC) in Figure 5. An FOC value equals to 1.0 indicates normal GJIC. The FOC value less than 1.0 indicates inhabitation. All experiments were done at least triplicate and the results were reported as mean ± standard deviation at the 95% confidence interval. When the 14 Hz ELF was applied vertically to the cell, we observed 20% inhibition of the GJIC within cells.

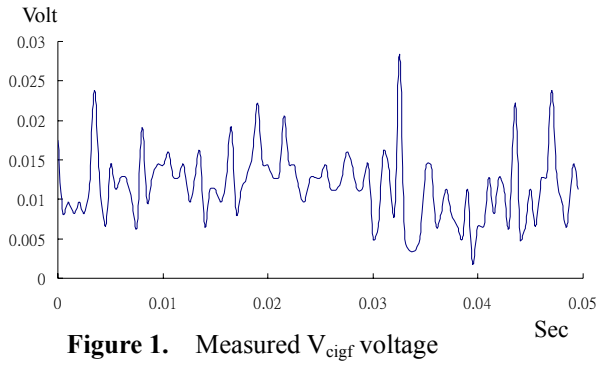

Figure 1. Measured V_{cigf} voltage

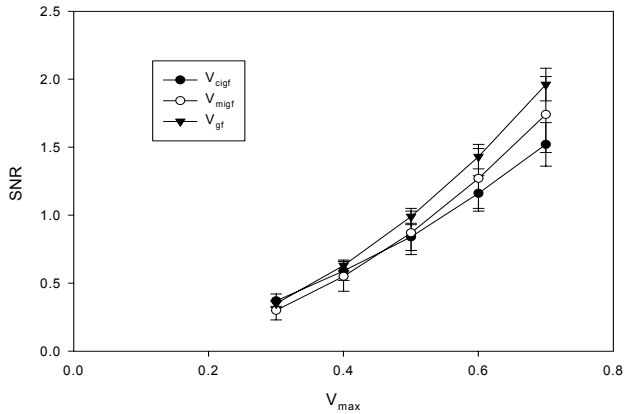

Figure 2. The signal to noise ratio fitting curve of the applied ELF test signal at 14 Hz. The intercept of the fitting curve is at 0.005 for V_{cigf}.

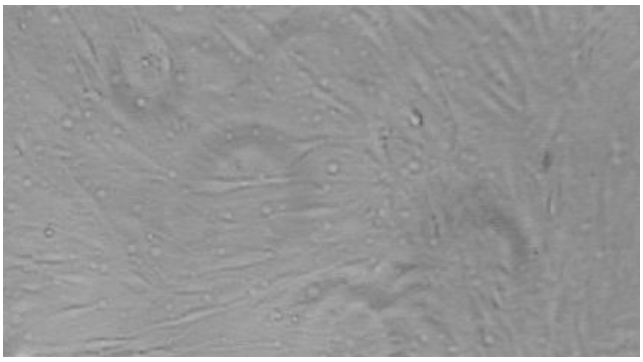

Figure 3a. The monolayer of the mouse osteoblast cells.

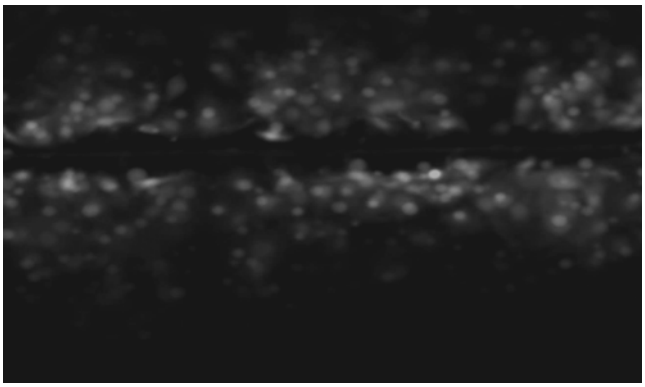

Figure3b. Fluoresce image of the GJIC of the cells in control.

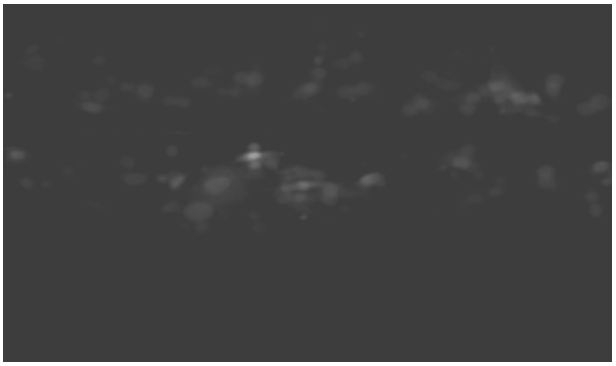

Figure 4. Fluoresce image of the GJIC of the cells under the exposure of 14 Hz ELF magnetic field.

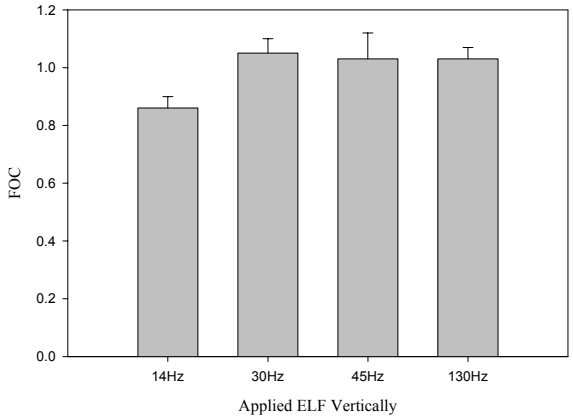

Figure 5. The FOC result of the GJIC Assay at 14 Hz ELF Exposure.

Discussion

The cell-induced current density in a 3.5 cm diameter cultured dish is proportional to the geomagnetic fluctuation, which fluctuates between 20 mGauss and 100 mGauss. The calculated intracellular/intercellular electrical current within a 3.5 cm diameter cell culture dish with confluent cells is about 10^{-9} Amp (Hart, 1996) under geomagnetic fluctuation. This report demonstrates test signals with defined amplitudes for detecting the signals buried in the V_{cigf} (t) and V_{migf} (t) providing evidence that V_{cigf} (t) affected the GJIC within cells. This study therefore, shows the mouse osteoblast cells could be a nonlinear system that can sense ELF signals and the intrinsic ELF signal is about 300 times less than the noise, which is ambiguous to obtain from direct measurement but can be detected by the help of V_{cigf} (t) with GJIC assay. Furthermore, Gap Junctional Intracellular Communication can be a target in which ELF acts on to cause the cell system responded. It is linked PDS to the degree modulation of GJIC within mouse osteoblast cells by using the amount of Lucifer yellow dye transfer area to indicate the modulation of GJIC due to the presence and absence of the ELF. The present results then indicated that the SNR analysis of the ELF signals buried in V_{cigf} can refer to the degree of modulation of GJIC within cells in the culture dish. Particularly, ELF at 14 Hz in cell system might be linked to ion cyclotron resonance (Liboff, 1991) and the calcium oscillation hypothesis (Fewtrell, 1993; Glaser, 1998).

Conclusion

We concluded that the cell induced electrical surface current on mouse osteoblast cells monolayer membrane attached to the cell culture plate under the influence of geomagnetic fluctuation created specific V_{cigf} (t). If we exposed the mouse osteoblast cells into intrinsic ELF at 14 Hz, it would modulate the GJIC within cells. Therefore, ELF 14 Hz is one of the intrinsic frequencies within mouse osteoblast cell system. Even though Adair (1991) criticized the resonance mechanisms, using the ion cyclotron resonance hypothesis, parametric resonance, electromagnetic coupling hypothesis, and the stochastic resonance mechanism may still validated to explain our experimental and calculated results. The major concern for this report, not only was the geomagnetic fluctuation affected cell system, but also we observed that the SNR of the intrinsic frequency, which power was so low as 200 to 300 times less than noise at 14 Hz. Since we exposed intrinsic frequency buried in the V_{cigf} and used the scrape load/dye transfer (SL/DT) technique to obtain the corresponding degree of modulation of GJIC, geomagnetic fluctuation and intrinsic ELF played the roles of adjusting GJIC within cells. Even we still question how cells can find the optimization between the V_{cigf} and the applied ELF signal that we see the GJIC modulation for cell system, we recognize geomagnetic fluctuation affected the intrinsic frequencies within the cells as well.

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