Bioelectromagnetics 39:249^252 (2018)

Letter to the Editor

Effects of Time Delays on Biological Feedback Systems and Electromagnetic Field Exposures

Frank Barnes* and Sahithi Kandala

University of Colorado, Boulder, Colorado

Keywords: biological; frequency; time; delays; responses

Biological systems contain a large number of signaling pathway and amplifying systems. Often these signaling systems operate in parallel and include both feedback and feed forward signals. An extensive review of how feedback loops shape cellular signals in space and time is presented by Brandman and Meyer [2008]. There are over 3,000 signaling proteins and over 15 s messengers that lead to hundreds of cell-specific signaling systems. These multiple feedback loops lead to a wide variety of responses including oscillations, bi-stability, and system stabilization. The multiple feedback loops often make it hard to separate cause and effect. For example, when we exercise, the metabolic rate is increased, which in turn increases the generation of reactive oxygen species (ROS), such as O_2^- and H_2O_2. The increased concentration of these molecules signals the generation of antioxidants that in normal circumstances return the concentration levels back to their normal resting values. The rate at which these ROS concentrations are returned to normal values is determined by the difference between the rate of generation and rate at which the antioxidants are generated, and convert the ROS molecules into other molecules such as H_2O and CO_2. There is a time delay between the increase in ROS concentrations and the return to normal levels. ROS radicals and H_2O_2 are of particular interest as they are both signaling molecules and have also been shown to cause damage such as aging, cancer, and Alzheimer's when the concentrations are elevated for extended periods of time [Droge, 2002]. Changes in concentrations of ROS molecules have been shown to have a wide range of both positive and negative effects on biological systems [Halliwell and Gutteridge, 2015]. Early works on the exposures of biological systems to microwaves show that even when the biological system was held at constant temperatures, there were changes in membrane resistance that differed from the first exposures to the second, and there was a time delay in the response [Arber and Lin, 1985a,b]. More recent works show that magnetic fields have been shown to modify ROS concentrations [Georgiou, 2010; Castello et al., 2014; Usselman et al., 2014, 2016].

Time delays in the response of biological feedback systems are common in many biological systems including those of the immune system and biological repair systems. In this paper, we will present a simple model based on an electronic operational amplifier with a time delay τ in the feedback loop that shows that by changing the frequency, phase, or pulse repetition rate of an externally applied signal, we can change the sign of the feedback and thus switch the gain of the overall amplifier from amplification to attenuation.

A simple circuit model for a feedback amplifier with time delay in the feedback is shown in Figure 1. With reference to Figure 1, the input of the amplifier $V_1(t)$ equal to the sum of the input voltage $V_s(t)$ and feedback from the output voltage at (t-τ) is

$$V_1(t) = V_s(t) + bV_o(t - \tau) \quad (1)$$

$$V_o(t) = A_o V_1(t) \quad (2)$$

Substituting Equation 2 into Equation 1 and eliminating $V_1(t)$ yields

Grant sponsors: National Science Foundation; grant number: 000416452; Milheim Foundation.

Conflicts of interest: None.

*Correspondence to: Frank Barnes, Electrical, Computer and Energy Engineering Department, University of Colorado, Campus Box 425, Boulder, CO 80309. E-mail: frank.barnes@colorado.edu

Received for review 20 October 2017; Accepted 14 January 2018

DOI: 10.1002/bem.22114
Published online 19 February 2018 in Wiley Online Library (wileyonlinelibrary.com).

© 2018 Wiley Periodicals, Inc.

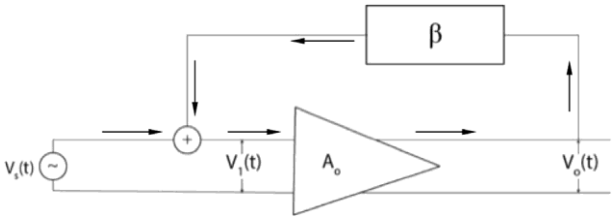

Fig. 1. A simple operational amplifier with a time delay t in feedback circuit b.

$$\frac{V_o(t)}{V_s(t)} = A_o \left(\frac{bA_o V_o(t - t)}{V_s(t)} \right) \quad (3)$$

Here, $V_o(t)$ is output voltage at time t, $V_s(t)$ is input signal at time t, b is feedback coefficient, A_o is gain of the amplifier, and $V_o(t\text{-}t)$ is output voltage at a time t earlier than t. A step function input to an amplifier with negative gain A_o and content feedback with a time delay t yields an output voltage that decays exponentially in steps with intervals of t. If the input signal is given by $V = V_{in} \cos(vt)$ and output signal is given by $V_o \cos(vt - u)$, where $u = vt$ and v is the angular frequency, the steady state equation can be rewritten as

$$A_f = \frac{V_o(t)}{V_s(t)} = \frac{A_o}{1 - bA_o \frac{\cos(vt - u)}{\cos vt}}$$

$$= \frac{A_o}{1 - bA_o(\cos u + \tan vt \sin u)} \quad (4)$$

From this equation it is easy to see that the sign of the feedback changes as the phase angle u changes. The term tan vt sin u varies from zero to plus or minus infinite with vt so that our overall gain A_f oscillates between zero and $A_f = \frac{A_o}{1 - bA_o \cos u}$. Thus, the response of our amplifier system is dependent on the angular frequency v and time delay t. If we examine the system at times when $vt = np$, the term $bA_o \cos(vt)$ changes sign with frequency and A_f will increase or decrease from the value for a system with zero-time delay with changes in frequency. When $bA_o \cos u = 1$, the system breaks into oscillation with no externally applied signal. A model that describes the effects of time delays in the control of frequency in biochemical oscillators that takes into account delayed negative feedback, sufficient "nonlinearity" of reaction kinetics, and proper balancing of time-scales of opposing chemical reactions is presented by Novak and Tyson [2008].

An example of the effects of a system-delayed feedback that leads to either amplification or attenuation of NAD(P)H, reactive oxygen, and nitric oxide oscillations, depending on the timing of the applied electric field stimulus for human neutrophils, is given by Rosenspire et al. [2005]. Thus, the system can be modeled as a feedback system with gain and a time delay. They show that electric fields as weak as 5×10^{-5} V applied at the minimum concentration and oscillating frequency of NAD(P)H concentrations leads to amplification. Figures 2 and 3 show the

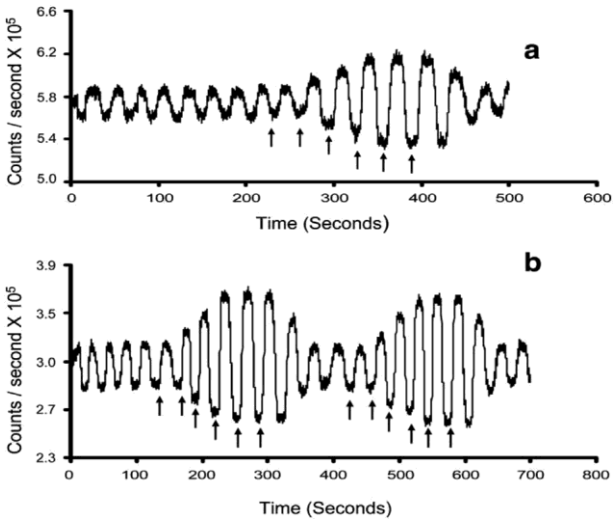

Fig. 2. NAD(P)H concentration in motile neutrophils is oscillatory, and amplitude of oscillation can resonate in the sense that amplitude increases with externally applied pulsed magnetic fields. NAD(P)H auto fluorescence was monitored with a photomultiplier and photomultiplier counts plotted for two different cells in a and b. Note the amplitude of the signal returns to its normal value when stimulus is removed [Rosenspire et al., 2005, Fig. 2].

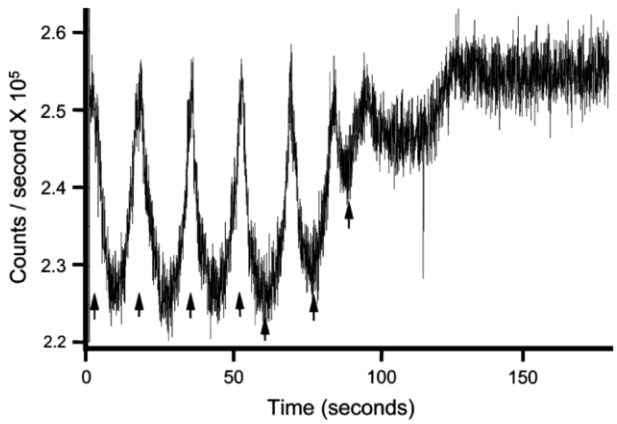

Fig. 3. Flavoprotein redox oscillations are inhibited by pulsed magnetic fields timed to coincide with minimal flavoprotein auto fluorescence, and they amplify oscillations when timed at minimums [Rosenspire et al., 2005, Fig. 11].

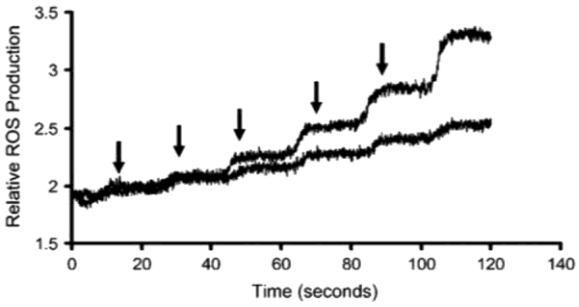

Fig. 4. Graph shows increase in production of hydrogen peroxide in response to pulsed magnetic field. Curve in the graph represents production of hydrogen peroxide in neutrophil, which was exposed to pulsed magnetic field at points represented by arrows. Lower curve is the production of hydrogen peroxide to which no magnetic pulse was applied [Rosenspire et al., 2005, Fig. 6].

period of oscillation was approximately 25 s. The electric fields were generated by time-varying magnetic fields or by applying a voltage between a pair of platinum electrodes. This corresponds to a period $t = 25$ s and a loop gain of $A_o b = 1$. The results are a function of multiple processes, time constants, and feedback loops.

From Figure 4 [Rosenspire et al., 2005, Fig. 6], the data are used to find A_o and b. In Table 1 are values of the data and calculated amplifier gain and feedback coefficient. The applied magnetic field pulse induces an electric field value of 8 mV/m, and the corresponding values for change in concentration of hydrogen peroxide are shown. From the previous mentioned values, we get A_o using Equation 2.

The obtained values for amplifier gain are 4×10^{-3}, 3.809×10^{-3}, 3.478×10^{-3}, 3.2×10^{-3}, and 2.80×10^{-3}, with respect to change in time. Each input pulse added to gain is in addition to the previous amplified value. The gain from successive input pulses decreases as we go forward in time and is likely to be the result of an additional negative feedback loop. Equation 4 for loop gain can be used to find b, since we have values of A_o and $A_o b = 1$.

Measurements of these parameters will vary from experimental system to system and with time with respect to the cell cycle. In the reactive oxygen antioxidant system you would want to measure the change in reactive oxygen without the antioxidant as a function of the applied magnetic field. This would give you a value for A_o. Time constants for various biological processes vary from less than a nanosecond to years.

It is to be noted that the diffusion time for small molecules across a cell is estimated to be in the range of hundredths of a second and, for proteins, in the vicinity of 100 s. Thus, periodic signal frequencies or pulse repetition rates ranging from hundreds of seconds to tens of seconds may lead to resonances associated with the transport of ions or molecules across cells. As the rate of generation of chemicals such as ROS change variations in the time delay for the generation of antioxidants, then the return of ROS concentrations to their normal value will also change. The resonance frequency for biological effects from magnetic fields to cause changes in ROS will change with the amplitude and frequency of the applied magnetic field. It is to be noted that there are many biological time constants in feedback loops regulating things like cell growth. We can expect signals such as modulated sine waves or pulses at different repetition rates containing more than one frequency to modify more than one biological process.

Overall, we know that there are many feedback and repair processes in biological systems. These feedback processes occur with time delays following a stimulus, and thus we can expect that the timing of a periodic stimulus can either lead to an amplified or attenuated response. Additionally, we can expect the responses of biological systems to be frequency-dependent. With knowledge of time constants for various biological and medical responses, we may be able to signal the systems to increase or decrease such things as cell growth rates or immune responses.

TABLE 1. The Table Shows the Input and Output of the System

Number of the pulse in the input sequence	Input voltage (mV)—$V_o(t)$/m	Output concentration (hydrogen peroxide concentration)— $V_1(t)$	Amplifier gain—A_o	Feedback coefficient (b)
1	8 mV	2	4×10^{-3}	250
2	8 mV	2.1	3.809×10^{-3}	262.536
3	8 mV	2.3	3.478×10^{-3}	287.5215
4	8 mV	2.5	3.2×10^{-3}	312.5
5	8 mV	2.85	2.80×10^{-3}	357.1428

The input is the electric field, in V/m in the system. The output of the system is proportional to the change in hydrogen peroxide concentration. The corresponding values of A_o and b are obtained.

ACKNOWLEDGMENTS

Many productive conversations with Rodolfo Bruzon, Hakki Gurhan, and Ben Greenebaum are much appreciated.

REFERENCES

Arber S, Lin JC. 1985a. Microwave-induced changes in nerve cells: Effects of modulation and temperature. Bioelectromagnetics 6:257–270.

Arber S, Lin JC. 1985b. Extracellular calcium and microwave enhancement of conductance in snail neurons. Radiat Environ Biophys 24:149–156.

Brandman O, Meyer T. 2008. Feedback loops shape cellular signals in space and time. Science 322:390–395.

Castello P, Hill I, Portelli L, Barnes F, Usselman R, Martino C. 2014. Inhibition of cellular proliferation and enhancement of hydrogen peroxide production in fibrosarcoma cell line by weak radio frequency magnetic fields. Biolectromagnetics 35:598–602.

Droge W. 2002. Free radicals in the physiological control of cell function. Physiol Rev 82:47–95.

Georgiou C. 2010. Non-thermal effects and mechanisms of interaction between electromagnetic fields and living matter. Section A: Oxidative stress-induced biological damage by low-level EMFs: Mechanisms of free radical pair electron spin-polarization and biochemical amplification. Eur J Oncol Library 5:63–113.

Halliwell B, Gutteridge J. 2015. Free Radicals in Biology and Medicine, 5th Edition. Oxford, United Kingdom: Oxford University Press. pp 1–30.

Novak B, Tyson J. 2008. Design principles of biochemical oscillators. Nat Rev Mol Cell Biol 9:981–991.

Rosenspire A, Kindzelskii A, Simon B, Petty H. 2005. Real-time control of neutrophil metabolism by very weak ultra-low frequency pulsed magnetic fields. Biophys J 88:3334–3347.

Usselman R, Hill I, Singel D, Martino C. 2014. Spin biochemistry modulates reactive oxygen species (ROS) production by radio frequency magnetic fields. PLoS ONE 9:1–12.

Usselman R, Chavarriaga C, Castello R, Procopio M, Ritz T, Dratz E, David J, Singel D, Martino C. 2016. The quantum biology of reactive oxygen species partitioning impacts cellular bioenergetics. Sci Rep 6, Article no. 38543.