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BEHAVIOR OF THE SQUARE WAVE IN BIOLOGICAL STRUCTURES

COMPORTAMENTO DA ONDA QUADRÁTICA EM ESTRUTURAS BIOLÓGICAS

COMPORTAMIENTO DE LA ONDA CUADRADA EN ESTRUCTURAS BIOLÓGICAS

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ABSTRACT
This study is to experimentally investigate the behavior of quadratic waves in biological structures. Transcutaneous frequency stimulation was regulated so that the system provided high frequency and low electrical voltage to observe possible changes in deeper layers of the anatomical structure analyzed. Electrodes were positioned from the dermis and probing electrodes were positioned at the subdermal, intradermal, skeletal muscle, periosteum, endosteum, and medulla levels. A function generator connected to a digital oscilloscope produced a square wave of 33 kHz at 10µs pulse speed and voltage of 16 volts DC. A voltage decay was observed as the signal reached deeper layers of different tissues. However, the frequency of 33 kHz remained uniform even at the bone marrow level. This observation opens the way for the application of the square electronic frequency as a stimulus for drug activation.

KEYWORDS: Electroceuticals. Bioelectronics. Square wave.

RESUMO
Este estudo tem por objetivo investigar experimentalmente o comportamento de ondas quadráticas em estruturas biológicas. A estimulação transcutânea de frequência foi regulada de modo que o sistema forneceu alta frequência e baixa tensão elétrica para observar possíveis mudanças nas camadas mais profundas da estrutura anatômica analisada. Os eletrodos foram posicionados a partir da derme e os eletrodos de sondagem foram posicionados nos níveis subdérmico, intradérmico, músculo esquelético, periósteo, endósteo e medula. Um gerador de funções conectado a um osciloscópio digital produziu uma onda quadrada de 33 kHz a 10µs de velocidade de pulso e tensão de 16 volts DC. Uma queda de tensão foi observada à medida que o sinal atingia camadas mais profundas de diferentes tecidos. Entretanto, a frequência de 33 kHz permaneceu uniforme mesmo no nível da medula óssea. Esta observação abre o caminho para a aplicação da frequência eletrônica quadrada como um estímulo para a ativação de drogas.

PALAVRAS-CHAVE: Eletrocêutica. Bioeletrônica, Onda quadrada.

RESUMEN
Este estudio pretende investigar experimentalmente el comportamiento de las ondas cuadráticas en estructuras biológicas. La estimulación transcutánea de frecuencia se reguló de forma que el sistema proporcionara una alta frecuencia y un bajo voltaje eléctrico para observar posibles cambios en las capas más profundas de la estructura anatómica analizada. Los electrodos se colocaron a partir de la dermis y los electrodos de sondeo se colocaron a nivel subdérmico, intradérmico, del músculo esquelético, del periostio, del endostio y de la médula. Un generador de funciones conectado a un osciloscopio digital produjo una onda cuadrada de 33 kHz a una velocidad de pulso de 10µs y un voltaje de 16 voltios DC. Se observó un descenso del voltaje a medida que la señal alcanzaba las capas más profundas de los diferentes tejidos. Sin embargo, la frecuencia de 33 kHz se mantuvo uniforme incluso a nivel de la médula ósea. Esta observación abre el camino para la aplicación de la frecuencia electrónica cuadrada como estímulo para la activación de fármacos.

PALABRAS CLAVE: Electrocéuticos. Bioelectrónica. Onda cuadrada.

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INTRODUCTION

We are fundamentally electronic beings. Our cells use ionic currents and electric fields to drive the transduction mechanism. Among numerous examples, it can be mentioned rhodopsin, a protein pigment found in retinal rods important for vision, which is activated by light that triggers the cascade of amplification signals that leads to a reduction in the cGMP gating of the Na+ channel. The change in the ionic current modifies the membrane potential of the rod and influences signaling to other cells in the visual cortex that eventually form an image in our brains. Another example is hearing. Air compressions impinge on the eardrum. The neuromotor ciliary kinetics of inner ear cells triggers the opening of ion channels for transduction of vibration into membrane potential, which converts into electrical signals that the brain interprets as noise. In simple terms, cells, tissues, and organs constantly send out electronic signals to maintain homeostasis, enable physiological functions, and respond to injury and infection. At the slightest signaling error, a multitude of pathologies can occur. The knowledge of electronic aspects of the human body leads to the search for methods that can help the correction of organic dysfunctions [(1) (2)].

Technically all organs and systems are regulated by the brain. Neural circuits communicate through electronic triggers at certain frequencies and wavelengths. Even the endocrine system is under the control of the central nervous system by a complex set of feedback mechanisms. Most drugs interact electronically with final (neural) or endocrine receptors. Therefore, Electroceuticals appears as a technology that aims to complement and eventually replace some drugs of a chemical nature with electronic stimuli (digital drugs) capable of creating a response in cellular ultrastructures [(2) (4)].

DEFINITION OF ELECTROCEUTICALS

It is a recently coined term for an ancient therapeutic modality that encompasses all of bioelectronic medicine. Basically, this refers to the electrical stimulation that can affect and/or modify body functions through *in situ* neural implants, such as cochlear implants, retinal implants or spinal cord stimulators for pain relief, but also cardiac pacemakers and implantable defibrillators. Recently, the field has expanded to include deep brain stimulation and electrical vagus nerve stimulation. Also called bioelectronics, the idea is that small electronic implants are capable of treating a wide range of chronic diseases, such as diabetes, asthma, airway obstruction, arthritis, hypertension and heart disease, as well as gastrointestinal problems [(5)].

MECHANISM OF ACTION

Briefly, the mechanism of action of electroceuticals is based on electrostimulation, whose access route is the vagus nerve (implant technique) [(3)].

This nerve is long and originates from the skull, precisely located behind the medulla oblongata - the structure that connects the brain with the spinal cord - it passes through the jugular foramen, the

neck, and thorax to the stomach. The first step in the development of this therapy is to accurately map the neural circuits associated with the disease and consequently its treatment [5].

ELECTRONIC INTERFERENCE

The existence of free ions, dipoles, and polarized molecules within the human body allows cells, tissues, and organs to generate and conduct electricity. Bioelectric voltage plays a vital role in regulating physiological processes. Based on this and on the complexity of vagal innervation, added to the production of electrical triggers from the neural network, sending electronic signals becomes a challenge, as it is assumed that such signals of endogenous and cellular nature flow through the body and generate a noisy electronic network difficult to control. A therapeutic electrical stimulus, for example, could suffer interference due to the organic electrical network itself, making the maneuver not fully effective [2].

MAIN GOAL

In situ implants for electrostimulation have become unquestionably indispensable in modern health. Functionality, miniaturization, biocompatibility, and/or biodegradability are the main engineering focuses for the development and clinical application of these respective implants. This technology is focused mainly on developing wearable/implantable energy devices and advanced conformable electrodes for efficient stimulation of targets such as organs and tissues. Electrodes deliver electrical signals to the target tissue/organ [4].

SPECIFIC OBJECTIVE

To present a possible alternative, non-invasive and economical evidence of using electronic frequency in the form of a square wave to stimulate the biological system.

MATERIALS AND METHODS

For the experiment, it was used a function generator of the Minipa brand, model MFG-4202A, according to the technical description:

- Waveforms: Sine, Square, and Triangular
- Frequency Range: 2Hz to 2MHz
- Output Impedance: 50Ω ± 10%
- Maximum Output Amplitude:>16Vpp open >8V with 50Ω load
- Amplitude Attenuation: 0dB, -20dB, -40dB
- Sine Wave Distortion: <2% (20Hz~20kHz)
- Square Wave Rise & Descent Time: <40ns

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• Triangular Wave Linearity: 98% (<100kHz) 95% (>100kHz)

• Operating Temperature: 0°C to 40°C

• Storage Temperature: -10°C to 50°C

• Relative Humidity: <80%

• Intern use

• Power: 110V/220V 60Hz

• Consumption: Less than 20W

This equipment provides flexible signals. With it, it is possible to simulate various types of waves such as sine, triangle, and quadratic waves over a wide range of time (0.01 Hz to 30 MHz), with amplitude and displacement control (offset) and constant voltage added to the signal. The synthesized digital circuit is capable of producing stable and accurate signals.

To control the generated signal, it was used a tablet-type digital oscilloscope, FNIRSI brand, according to the technical description:

• Analog bandwidth: 100mhz * 2

• Number of channels: 2 channels

• Maximum real-time sampling rate: 1gsa/s

• Vertical sensitivity: 50 mv/div ~ 500 v/div

• Horizontal time-based range: 50s/div 1010ns/div

• Maximum test voltage: 40v (1x probe), 400v (10x probe)

• Storage Depth: 240 kbit

• Input resistance: 1m

• adc accuracy: 8bit

• Coupling mode: ac/dc

• Shooting mode: single, normal, automatic

• Trigger edge: rising edge/falling edge

• External trigger voltage 0 - 40 v

• Display: 7 inch - 800*480

• Operation: capacitive touch screen

• Extension ports: USB image export

• Power supply: 6000mAh lithium battery

Electrodes for the incident wave (EOI):

Reusable electrodes in alpaca metal, specific for monitoring ECG procedures, with pin terminations.

Resulting Pickup Electrodes (ECR):

Copper wire as per specification:

Table 1. Copper electrode specification

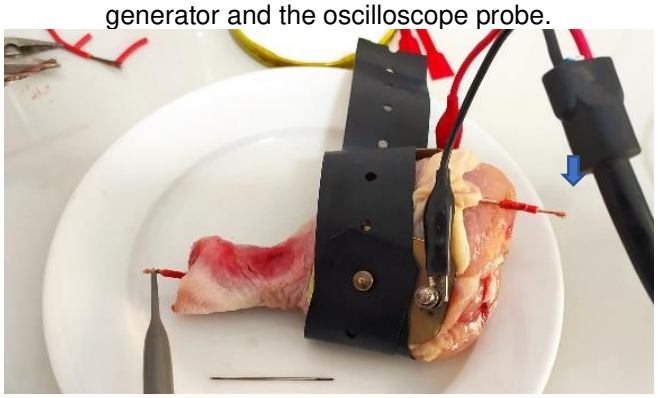
AWG	Diameter (mm)	Circular section (mm^2)	Resistance (Ohms/Km)
4	5.189	21.15	0.80

Biological material probed

The experiment used the leg of a galliform bird of the species *Gallus domesticus*, collected from a slaughterhouse.

First, the ECRs were inserted aiming at contact with the respective histological areas to be studied: subdermis, intradermis, skeletal muscle, periosteum, endosteum, and medulla. Then, the dermis of the anatomical structure was cleaned with alcohol for EOI positioning. The fixation was done through a strip of insulating polymer based on cis-polyisoprene.

Figure 1. The anatomical structure of a bird connected to the EOI and connected to the incident wave generator and the oscilloscope probe.



It is possible to verify the presence of the electrode at the subdermal level as indicated by the arrow.

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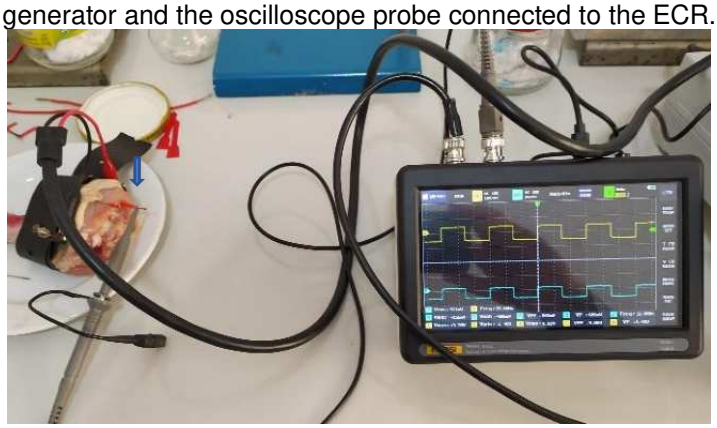
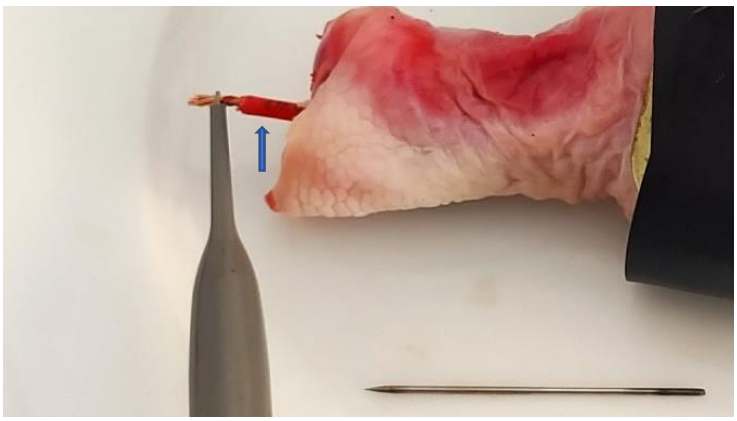
Figure 2. Bird anatomical structure connected to the EOI and connected to the incident wave generator and the oscilloscope probe connected to the ECR.



The probing electrode is intramuscularly inserted as indicated by the arrow.

Figure 3. Anatomical structure probed by the probe connected to the ECR electrode.



ECR electrode inserted in the bone marrow region.

Signal regulation

The equipment was adjusted to the following parameters:

• Square wave

• Amplitude: 10 v/div

• Pulse speed: 10 µs

• Frequency: 33 kHz

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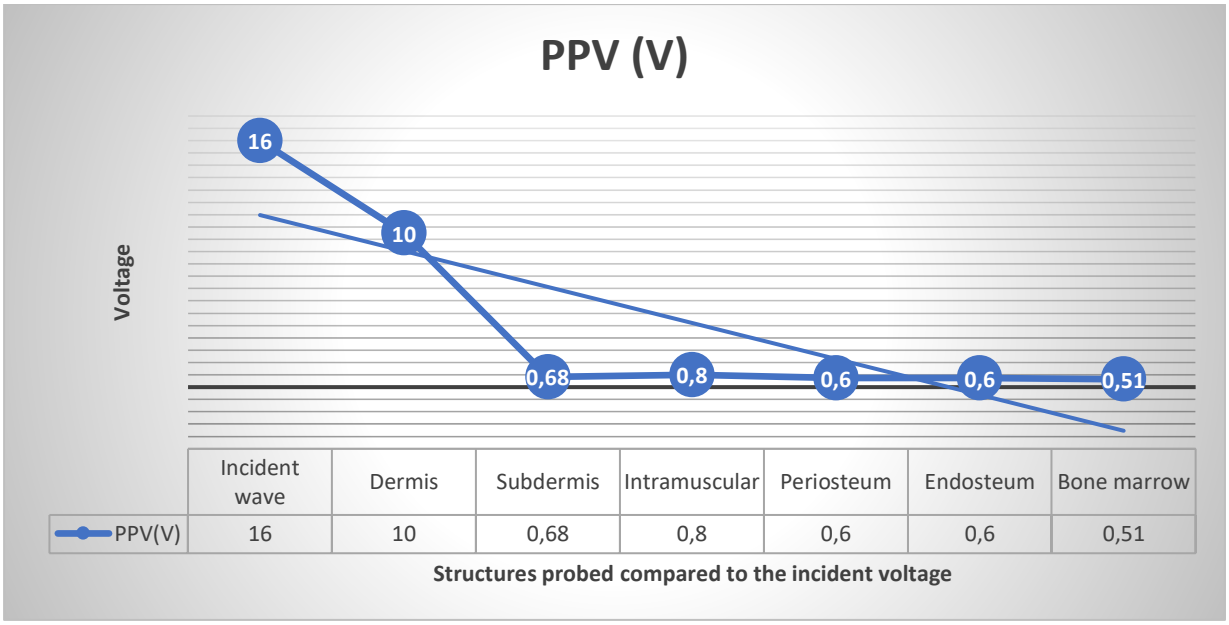
RESULTS

Immediately after triggering the incident wave, the data collected by the resulting pickup electrodes (ECR) showed voltage decay:

Table 2. Incident voltage and probed structures (VPP - Peak-to-peak voltage)

Probing	VPP (V)
Incident	16
Dermis	10
Subdermis	0.68
Intramuscular	0.8
Periosteum	0.6
Endosteum	0.6
Medulla	0.51

Graph 1. The trend line of probed structures



The trend line of the graph indicates that as it reaches deeper regions in the anatomical structure, there is a considerable loss of voltage. When comparing the initial voltage of 16 volts with the medullary region, there is a decrease of 96.81% in electrical conductivity.

However, this is not often applied:

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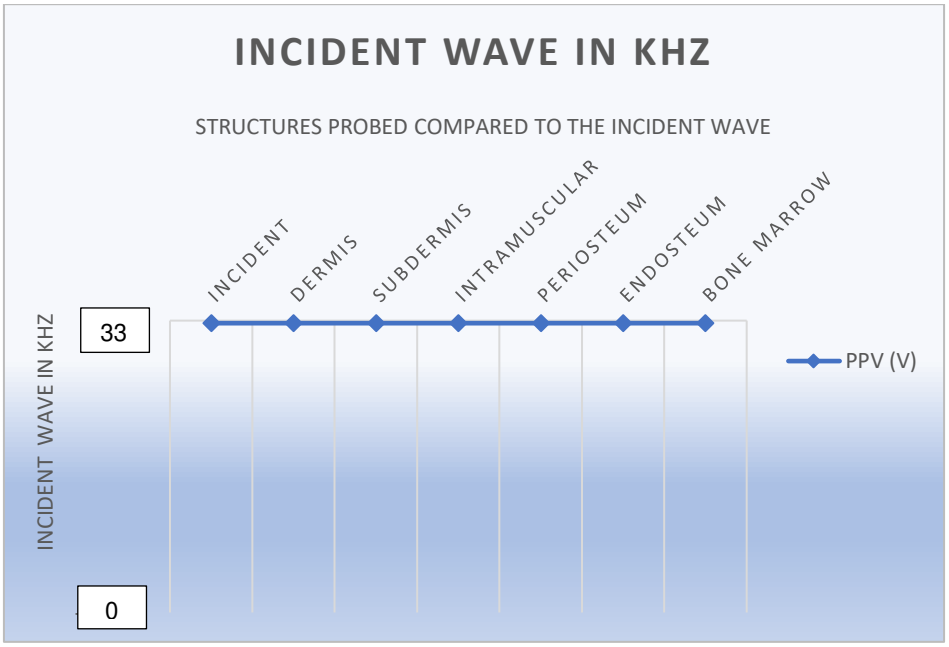
Table 3. Incident frequency and sounded structures

Probing	kHz
Incident	33
Dermis	33
Subdermis	33
Intramuscular	33
Periosteum	33
Endosteum	33
Bone marrow	33

Graph 2. Incident wave in kHz and sounded structures

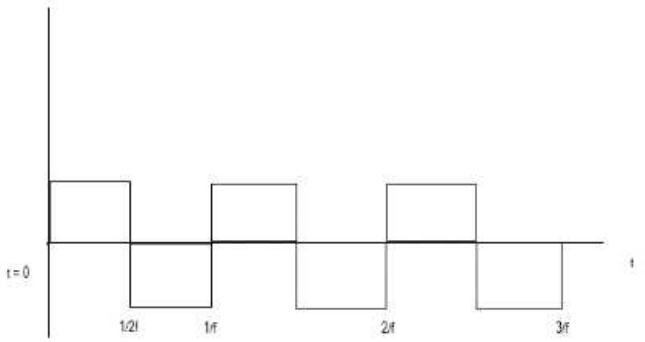


Despite the decay of the incident voltage, the frequency of 33 kHz reaches all structures equally, without suffering distortions.

DISCUSSION

Electromagnetic radiation is the result of changing the speed of moving charges. Propagation occurs at the speed of light. The square wave was chosen for this experiment taking into account its wide range of harmonics and its effect between the high and low levels, producing the hammer effect. The times it takes for the signal to rise from the low level to the high level and back are called the rise time and fall time, respectively. [5] [8]

Figure 4. Square wave at 33 kHz



The square wave acts as an agitator of nanometric structures. In this case, biological tissues are naturally overdamped. The elastic reactance of the cell membrane is an example. The waveform may never actually reach the theoretical high and low levels, and if the system is underdamped, it will oscillate between high and low levels before stabilizing [9].

A priori, according to graph 2, it can be deduced that the frequency stabilized at 33 kHz would represent that biological structures are invisible to this wave. However, by converting the respective frequency into nanometers, the effect reaches structures in the order of 7.1 nm.

$$n_{1,2} = \frac{n_1}{n_2} = \frac{\frac{c}{v_1}}{\frac{c}{v_2}} = \frac{v_2}{v_1}$$

1 = Propagation of light in the medium without resistance

n1.2 = Refractive index of medium 1 in relation to medium 2

v1 and v2 = Speed of light in media 1 and 2

The density of blood and lymph = 1.28 g/cm3

$$\frac{v}{D}$$

V = speed of light

D = blood/lymph density

$$\frac{300{,}000{,}000}{1.28} = 234{,}375{,}000 m/s$$

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The speed of light in a blood medium slows down. From the result, the frequency ratio is obtained, in this case, 33,000 Hz = 33 kHz

$$\frac{v}{f}$$

V = speed of light in blood or lymph

f = Frequency used

$$\frac{234{,}375{,}000}{33{,}000} = 7102.27$$

The result is in nanometers = 7.1, that is, the frequency of 33 kHz is capable of resonating with structures in this dimension. It is important to note that resonance refers to the vibrating system that induces another system to oscillate. On the scale of 7.1 nanometers, this would equate to the cell membrane thickness of bacteria, algae, fungi, protozoa, and animals. As the frequency increases, the nanometer scale decreases. Consequently, even smaller structures can be achieved.

Thus, it is possible to speculate that the application of ultrashort pulses (hammer effect), characteristic only of the quadratic wave, may cause reactions not only at the membrane level but also in any other nanostructure, such as ion channels of the order of 5 nm, which could be resonated at a frequency of 47 kHz.

Kilohertz	Nanometer Scale
33	7.1
35	6.7
38	6.2
42	5.6
47	5
55	4.3
60	3.9
65	3.6
80	2.9
90	2.6
115	2

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CONCLUSION

The experiment indicates that kilohertz pulses can reach cellular structures and their external and internal substructures and therefore, possibly alter their functions. This is independent of the applied voltage. Even molecules could be stimulated. In this case, the frequency would be the acting agent. Since the typical loading time constants of cells with dimensions of 10 µm are on the order of one hundred nanoseconds, submicrosecond electrical pulses are expected to cause different effects. New experiments must be conducted to evaluate the respective behaviors at the nanometer/micrometer level and, consequently, at the tissue level as a whole [6].

Speculatively, it can be considered the application of the administration of pulse electric fields to activate molecules with therapeutic or prophylactic potential inside cells. Activation would take place through specific frequencies. In the case of chemotherapeutic agents, the influx or efflux of the drug through the receptors could be controlled frequently. The use of pulses would assist in this process, giving the agent a more direct path to the cytosol. Pulsatile electronic waves would allow access to the cytoplasmic target for drugs that could only cross the cytosol very poorly. It is important to emphasize that the frequency used is not ionizing, since it would have to be in the order of more than 20 MHz [7].

There are some research possibilities to be developed in the future:

- ✓ Use of electronic frequency to activate a drug;
- ✓ Acting as a cell stimulator to accelerate tissue regeneration processes;
- ✓ Activation of defective cell receptors by frequency resonance;
- ✓ Adjuvant in antibiotic treatments.

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