IMPLEMENTATION OF TRANSCRANIAL MAGNETIC STIMULATION FOR EXPERIMENTAL STUDIES OF "EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS"

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1 – Background

This essay is included in a wider project which objective is to **determine in vivo**, the **transmission speed** of motor evoked potentials (MEP) in nerve tract, and thus to research the **condition of the myelin sheath** as well as the behavior of nerve conduction in **an animal model** of demyelinating diseases.

In Nerve Conduction Studies (NCS), time (milliseconds) measures as well as amplitude (millivolts) of response, are taken.

The latency is the time it takes to the MEP to travel from the stimulation point in the motor cortex to the recording point in the muscles.

Speed can be calculated taking into account the distance between the stimulating and recording points, and the latency.

The "Transcranial Magnetic Stimulation (TMS)" (1÷3, 7) has been shown as an effective technique for artificially generate MEP and through them to study the physiological characteristics of demyelinating disorders.

In our project a magnetic pulse generator at small scale has been specifically developed for this purpose.

Once this developed prototype is tuned and scaled up to the parameters needed to elicit an action potential, it will be applied on the "Experimental Autoimmune Encephalomyelitis" animal model.

1.1 Anatomical and Physiological aspects of nerve conduction

1.1.1 Basic concepts of neuron and glia

A neuron is a cell that processes and transmits information through electrical and chemical signals, it is the main signaling unit of the Nervous System.

These electrical signals, called **action potentials**, are rapid, transient, all-or-none nerve impulses, with an amplitude of 100 mV and a duration of about 1 ms.

The chemical and electrical signals are transmitted via the synapses which are present in Axon and Dendrite terminations.

The signals that neurons convey are identical, whatever it is the transmitted information (vision, odor, sound, etc.).

The information conveyed by an action potential is determined not by the form of the signal but by the pathway the signal travels in the brain.

To increase the speed by which action potentials are conducted, large axons are wrapped in a fatty, insulating sheath of myelin. The sheath is interrupted at regular intervals by the nodes of Ranvier. It is at these uninsulated spots on the axon that the action potential becomes regenerated.

The neurons are electrically excitable cells and connect to each other to form neural networks. Neurons are the core components of the nervous system, which includes the brain, spinal cord, and peripheral ganglia.

There are more than 10^{11} neurons in the human body that can be classified into many different types according to a wide variety of shape, size and electrochemical properties, and can perform a high diversity of functions.

Sensory neurons for example respond to different type of stimulus: heat, sound, light touch, and many other affecting cells of the sensory organs.

A typical neuron is divided into four parts: the soma, dendrites, axon and presynaptic terminals. The soma is the cell body, it is usually compact. The axon and dendrites are filaments that extrude from it. Dendrites typically branch profusely, getting thinner with each branching, and extending a few hundred micrometers from the soma.

An axon is a special cellular extension that arises from the cell body and travels – sometimes- a long distance (it could be longer than 1 meter in humans).

A neuron can have many dendrites but just one axon. The axon can give rise to hundreds of branches. Unlike dendrites, an axon usually maintains the same diameter as it extends.

The neurons are organized in a wide network that allows to perform very complex functions. Even if their properties are similar, neurons can produce very different actions thanks to the way they are connected with each other and with sensory receptors and muscles.

It is interesting to take into account four basic features of the nervous system:

1. The mechanisms by which neurons produce signals.
2. The patterns of connections between nerve cells.
3. The relationship between different patterns of interconnection and different types of behavior.
4. The means by which neurons and their connections are modified by experience.

Glial cells far outnumber neurons—there are between 10 and 50 times more glia than neurons in the central nervous system of vertebrates.

Glial cells act supporting and surrounding the cell bodies, axons, and dendrites.
As far as is known, glia cells are not directly involved in information processing, but they have other vital roles:

1. To provide the brain structure that supports neurons
2. To electrically insulate the axons thanks to myelin sheaths produced by two type of glial cells: Oligodendrocytes and Schwann cells
3. To remove debris after injury or neuronal death
4. To maintain homeostasis in its surroundings
5. To promote efficient signaling between neurons, e.g. taking up chemical transmitters released by neurons during synaptic transmission
6. To guide the migration of neurons and direct the outgrowth of axons
7. Actively regulating the properties of the presynaptic terminal
8. To form an impermeable lining in the brain's capillaries and venules— the blood-brain barrier—thus preventing toxic substances in the blood from entering the brain
9. Apparently releasing growth factors and otherwise helping nourish nerve cells
10. To supply nutrients and oxygen to neurons

1.1.2 Motor pathway

The motor systems of the brain and spinal cord allow us to maintain balance and posture, to move our body, limbs, and eyes, and to communicate through speech and gesture. In contrast to sensory systems, which transform physical energy into neural signals, motor systems produce movement by translating neural signals into contractile force in muscles.
The motor system is the part of the central nervous system that is involved with movement. It consists of the **pyramidal** and **extrapyramidal system**.
The **motor pathway** also called **pyramidal tract** or the **corticospinal tract** start in the motor center of the cerebral cortex.
The motor impulses originates in the giant pyramidal cells or Betz cells of the motor área.
The pyramidal tracts refers to both the **corticospinal** and **corticobulbar** tracts.
The corticospinal tract conducts impulses from the brain to the spinal cord. It contains mostly axons originated from the motor cortex. The corticospinal tract also contains the Betz Cell that are not found in any other region of the body. The corticospinal tract is

concerned specifically with discrete voluntary skilled movements, such as precise movement of the fingers and toes.
The corticobulbar tract carries information to motor neurons of the cranial nerve nuclei, rather than the spinal cord.
Lower motor neurons (LMNs) are the motor neurons connecting the brainstem and spinal cord to muscle fibers, bringing the nerve impulses from the upper motor neurons out to the muscles. A lower motor neuron's axon terminates on an effector (muscle).
Upper motor neurons are motor neurons that originate in the motor region of the cerebral cortex or the brain stem and carry motor information down to the final common pathway, that is, any motor neurons that are not directly responsible for stimulating the target muscle. The main effector neurons for voluntary movement lie within layer V of the primary motor cortex and are called Betz cells. The cell bodies of these neurons are some of the largest in the brain, approaching nearly 100μm in diameter.

1.1.3 Motor neurons and Cell excitability

Motor neurons (or motoneurons) is the type of neuron specifically in charge of transmission of motion signals. They receive signals from the brain and spinal cord, cause muscle contractions, and affect glands.
Motor neuron classically applies to neurons located in the Central Nervous System (CNS) that project their axons outside the CNS and directly or indirectly control muscles.
A typical neuron possesses a cell body (often called the soma), dendrites, and an axon. Dendrites are thin structures that arise from the cell body, often extending for hundreds of micrometers and branching multiple times, giving rise to a complex "dendritic tree".
The cell body of a neuron frequently gives rise to multiple dendrites, but never to more than one axon, although the axon may branch hundreds of times before it terminates.

Information proceeds from the cell body unidirectionally over the synapse, first along the axon and then across the synapse to the next nerve or muscle cell.
At the majority of synapses, signals are sent from the axon of one neuron to a dendrite of another. The part of the synapse that is on the side of the axon is called the presynaptic terminal; that part on the side of the adjacent cell is called the postsynaptic terminal. Between these terminals, there exists a gap, the synaptic cleft, with a thickness of 10 − 50 nm. The fact that the impulse transfers across the synapse only in one direction, from the

presynaptic terminal to the postsynaptic terminal, is due to the release of a chemical transmitter by the presynaptic cell.
This transmitter, when released, activates the postsynaptic terminal.
The synapse between a motor nerve and the muscle it innervates is called the neuromuscular junction.

Cell excitability:

All neurons are electrically excitable, maintaining voltage gradients across their membranes by means of metabolically driven ion pumps, which combine with ion channels embedded in the membrane to generate intracellular-versus-extracellular concentration differences of ions such as sodium, potassium, chloride, and calcium. The flow of these ions forms the basis of bioelectric phenomena.
Changes in the cross-membrane voltage can alter the function of voltage-dependent ion channels.

If the voltage changes by a large enough amount, an all-or-none electrochemical pulse called an action potential is generated, which travels rapidly along the cell's axon, and activates synaptic connections with other cells when it arrives.
Synaptic signals from other neurons are received by the soma and dendrites; signals to other neurons are transmitted by the axon. A typical synapse, then, is a contact between the axon of one neuron and a dendrite or soma of another. Synaptic signals may be excitatory or inhibitory. If the net excitation received by a neuron over a short period of time is large enough, the neuron generates a brief pulse called an action potential, which originates at the soma and propagates rapidly along the axon, activating synapses onto other neurons as it goes.

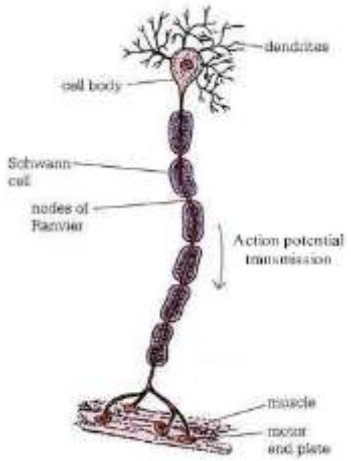


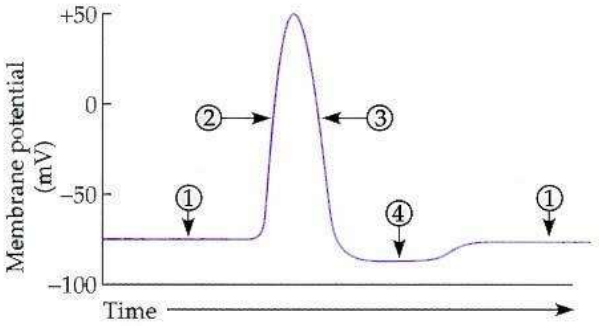
Fig.1 Motoneuron

Every type of cell has a voltage difference between inner and outer parts of its membrane. Both, nerve and muscle cells are excitable, their cell membrane can produce electrochemical impulses and lead them along the membrane.

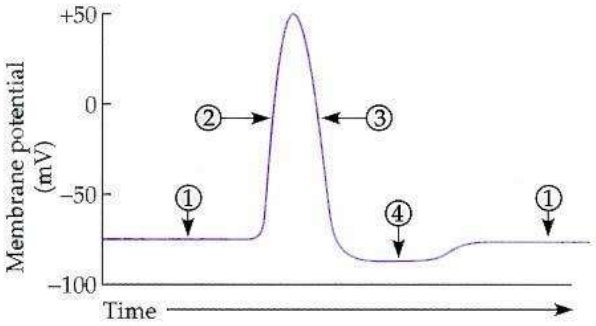
In muscle cells, these electrical phenomena are also associated with the contraction of the cell.

The transmembrane voltage (Vm) of an excitable cell can be defined as the potential difference between membrane's inner surface potential (Vi) and the outer (Vo),

$$Vm = (Vi) - (Vo).$$



Sodium ions are rushing in at the area labeled 2 (Depolarization)



Repolarization is labeled 3 (potassium ions are rushing out)

Fig 2. Membrane potential

Transmembrane potential fluctuates according to Fig.3.

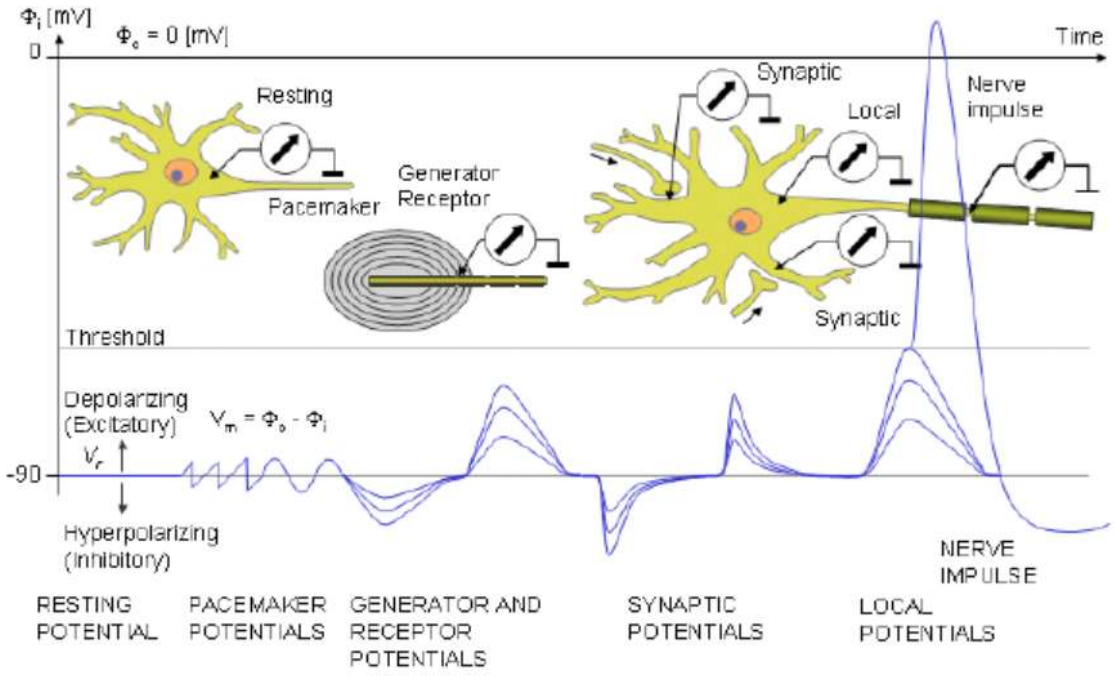


Fig 3. Transmembrane potentials according to T. H. Bullock (4).

Myelin sheath

Myelin is a protein which has electrical insulating properties.

The axon may be covered with an insulating layer called the myelin sheath.

The myelin sheath is a membrane spirally wrapped around the axon formed by proccesses of olygodendrocytes (in CNS) or Schwann cells (in Peripheral Nervous System). In myelinated axons action potential jumps from node to node of Ranvier increasing the conduction speed through the axon.

Muscle cell

There are three types of muscles in the body: smooth, cardiac and striated.
Smooth and cardiac muscles are involuntary, striated instead respond to motion orders carried by networks of motoneurons.
Striated muscles are formed from a large number of muscle fibers that range in length from 1 to 40 mm and in diameter from 0.01 to 0.1 mm.

Each fiber forms a (muscle) cell and is distinguished by the presence of alternating dark and light bands.
The striated muscle fiber corresponds to an unmyelinated nerve fiber but is distinguished electrophysiologically from nerve by the presence of a periodic transverse tubular system (TTS), a complex structure that, in effect, continues the surface membrane into the interior of the muscle.
Propagation of the impulse over the surface membrane continues radially into the fiber via the TTS, and forms the trigger of myofibrillar contraction. The presence of the TTS affects conduction of the muscle fiber so that it differs (although only slightly) from propagation on an unmyelinated nerve fiber.

1.1.4 Activation of nerve cell. Physiological basis of Action Potential

If a nerve cell is stimulated, the transmembrane voltage necessarily changes. The stimulation may be excitatory (i.e., depolarizing; characterized by a change of the potential inside the cell relative to the outside in the positive direction, and hence by a decrease in the normally negative resting voltage) or inhibitory (i.e., hyperpolarizing, characterized by a change in the potential inside the cell relative to the outside in the negative direction, and hence by an increase in the magnitude of the membrane voltage).
After stimulation the membrane voltage returns to its original resting value.

If the membrane stimulus is insufficient to cause the transmembrane potential to reach the threshold, then the membrane will not activate. The response of the membrane to this kind of stimulus is essentially passive.

If the excitatory stimulus is strong enough, the transmembrane potential reaches the threshold, and the membrane produces a characteristic electric impulse, the nerve impulse. This potential response follows a characteristic form regardless of the strength of the trans-threshold stimulus. It is said that the action impulse of an activated membrane follows an all−or−nothing law. An inhibitory stimulus increases the amount of concurrent excitatory stimulus necessary for achieving the threshold (Fig 4). The electric recording of the nerve impulse is called the "**action potential**".

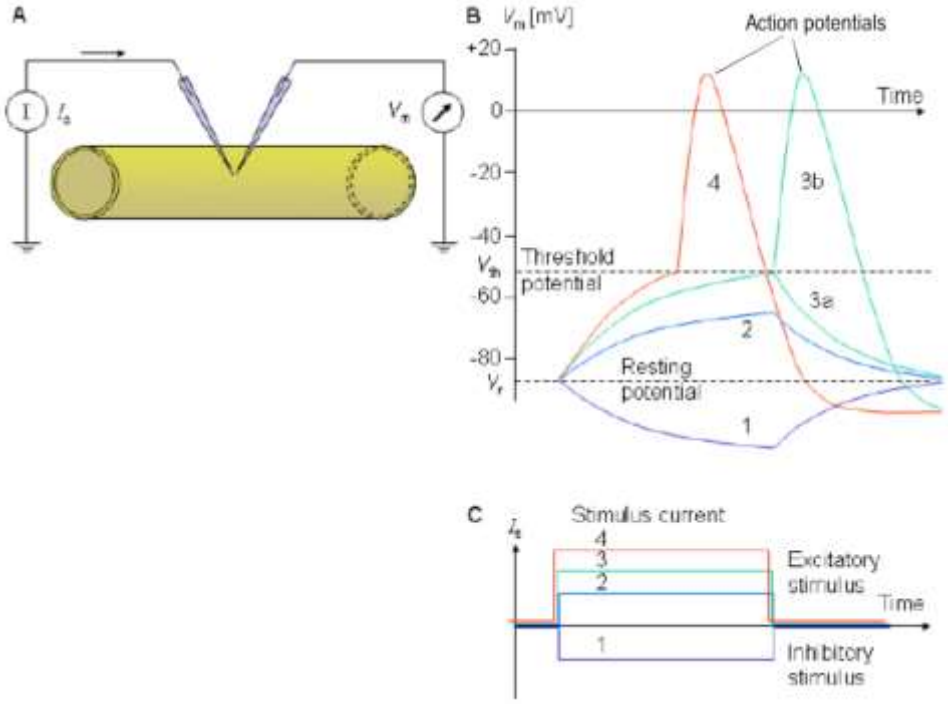


Fig. 4. (A) Experimental arrangement for measuring the response of the membrane potential; (B) Inhibitory (1) and excitatory (2, 3, 4) stimuli. Ref. (4).

When the stimulus is higher than the threshold potential, an action potential occurs.

Mechanism of activation

The concentration of sodium ions (Na+) is about 10 times higher outside the membrane than inside, whereas the concentration of the potassium (K+) ions is about 30 times higher inside as compared to outside.

When the membrane is stimulated so that the transmembrane potential rises about 20 mV and reaches the threshold − that is, when the membrane voltage changes from −70 mV to about −50 mV (these are illustrative and common numerical values) − the sodium and potassium ionic permeabilities of the membrane change.

The sodium ion permeability increases very rapidly at first, allowing sodium ions to flow from outside to inside, making the inside more positive. The inside reaches a potential of about +20 mV. After that, the more slowly increasing potassium ion permeability allows potassium ions to flow from inside to outside, thus returning the intracellular potential to its resting value.

The maximum excursion of the membrane voltage during activation is about 100 mV; the duration of the nerve impulse is around 1 ms.

While at rest, following activation, the Na−K pump restores the ion concentrations inside and outside the membrane to their original values.

Whether an excitatory cell is activated depends largely on the strength and duration of the stimulus. The neurons can be activated by a short, strong stimulus or a longer, weaker stimulus. The curve illustrating this dependence is called the strength−duration curve (fig.5).

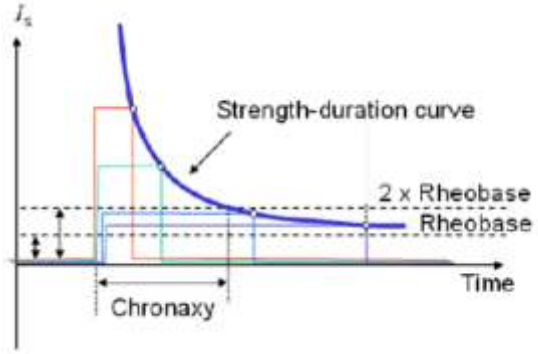


Fig 5 Strength−duration curve-(4).

The strength−duration curve describe the minimum combinations of strength and duration just needed to produce the activation. The smallest current adequate to initiate activation is called the rheobasic current or rheobase.

Accommodation and habituation denote the adaptation of the cell to a continuing or repetitive stimulus, this is characterized by a rise in the excitation threshold.

Facilitation denotes an increase in the excitability of the cell; correspondingly, there is a decrease in the threshold.

Latency denotes the delay between application of a stimulus pulse and the beginning of the activation.

Once activation has been initiated, the membrane is insensitive to new stimuli, no matter how large the magnitude. This phase is called the absolute refractory period. Near the end of the activation impulse, the cell may be activated, but only with a stimulus stronger than normal. This phase is called the relative refractory period.

The activation process encompasses certain specifics such as currents, potentials, conductivities, concentrations, ion flows, and so on. The term **action impulse** describes the whole process.

1.1.5 Conduction of nerve pulses in an Axon

It is known from Huxley and Hodgkin (Ref. 5) that the propagation of impulses in giant axons of squid is an all-or-none event resulting in an **advancing membrane potential change** along the fiber cable structure thereby stimulating adjacent, initially quiescent membrane.

The resting negativity of the fiber interior has been attributed to a selective permeability of the membrane to potassium relative to sodium, and the greater intracellular relative to extracellular potassium concentration.

The action potential might then result from a generalized increase in membrane permeability to all ions in response to alteration of the internal potential beyond a threshold level, collapsing this potential to zero.

An increased membrane permeability had indeed been demonstrated by membrane impedance measurements by a high-frequency alternating current bridge (5).

However, Hodgkin and A.F. Huxley's intracellular recordings of the action potential demonstrated that the membrane potential becomes substantially positive (4). This observation was to lead eventually to the current view that this reflects an increased permeability specific for sodium ions, which would diffuse inwards carrying a positive charge.

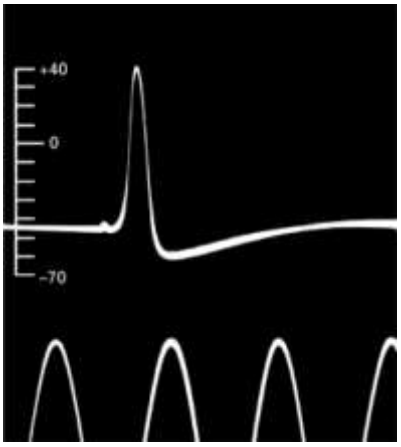


Fig 6. Intracellular recording of the squid giant axon action potential.

The activation propagates in an axon as an unattenuated nerve impulse.
Activation in the form of the nerve impulse (action potential) is first seen in the root of the axon − the initial segment of the axon- from there it propagates along the axon.
The conduction velocity depends on the electric properties and the geometry of the axon.
An important physical property of the membrane is the change in sodium conductance due to activation. The higher the maximum value achieved by the sodium conductance, the higher the maximum value of the sodium ion current and the higher the rate of change in the membrane voltage. The result is a higher gradient of voltage, increased local currents, faster excitation, and increased conduction velocity. The decrease in the threshold potential facilitates the triggering of the activation process.
The capacitance of the membrane per unit length determines the amount of charge required to achieve a certain potential and therefore affects the time needed to reach the threshold. Large capacitance values, with other parameters remaining the same, mean a slower conduction velocity.
The velocity also depends on the resistivity of the medium inside and outside the membrane since these also affect the depolarization time constant. The smaller the resistance, the smaller the time constant and the faster the conduction velocity. The temperature greatly affects the time constant of the sodium conductance; a decrease in temperature decreases the conduction velocity.
A myelinated axon (surrounded by the myelin sheath) can produce a nerve impulse only at the nodes of Ranvier. In these axons the nerve impulse propagates from one node to another as illustrated in Figure 7. Such a propagation is called saltatory conduction.

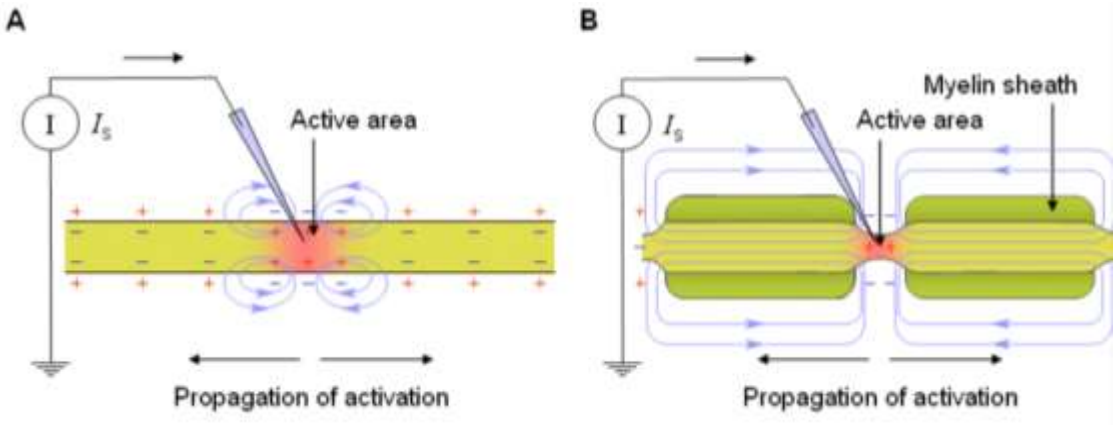


Fig. 7. Conduction of a nerve impulse in a nerve axon.
(A) continuous conduction in an unmyelinated axon;
(B) saltatory conduction in a myelinated axon.

Such a propagation is called saltatory conduction.
The membrane capacitance per unit length of a myelinated axon is much smaller than in an unmyelinated axon. Therefore, **the myelin sheath increases the conduction velocity** (17). The resistance of the axoplasm per unit length is inversely proportional to the cross−sectional area of the axon and thus to the square of the diameter. The membrane capacitance per unit length is directly proportional to the diameter.
Because the time constant formed from the product controls the nodal transmembrane potential, it is reasonable to suppose that the velocity would be inversely proportional to the time constant.
On this basis the conduction velocity of the myelinated axon should be directly proportional to the diameter of the axon. This is confirmed in Figure 8, which shows the conduction velocity in mammalian myelinated axons as linearly dependent on the diameter.
The conduction velocity in myelinated axon has the approximate value shown:
v = 6d , where v = velocity [m/s] and d = axon diameter [μm]

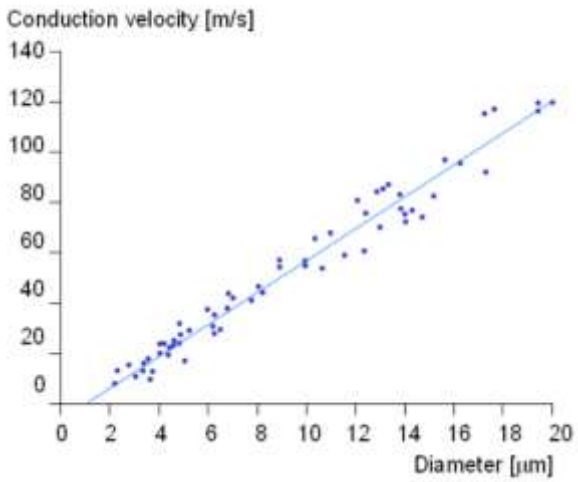


Figure 8. Experimentally determined conduction velocity of a nerve impulse in a mammalian myelinated axon as a function of the diameter (4).

1.1.6 Origin of the resting voltage in the neuron

The resting voltage of a nerve cell denotes the value of the membrane voltage (difference between the potential inside and outside the membrane) when the neuron is in the resting state in its natural, physiological environment.

Physiological basis of the resting voltage and the sub-threshold response of an axon to electric stimuli from a quantitative perspective:

The membrane plays an important role in establishing the resting and active electric properties of an excitable cell, through its regulation of the movement of ions between the extracellular and intracellular spaces.

The ease with which an ion crosses the membrane, namely the membrane permeability, differs among ion species; this selective permeability will be seen to have important physiological consequences. Activation of a cell affects its behavior by altering these permeabilities.

Another important consideration for transmembrane ion movement is the fact that the ionic composition inside the cell differs greatly from that outside the cell. Consequently, concentration gradients exist for all permeable ions that contribute to the net ion movement or flux.

One consequence of this ion flow is the tendency for ions to accumulate at the inner and outer membrane surfaces, a process by which an electric field is established within the membrane. This field exerts forces on the ions crossing the membrane since the latter carry

an electric charge. Thus to describe membrane ion movements, electric−field forces as well as diffusional forces should be considered. Equilibrium is attained when the diffusional force balances the electric field force for all permeable ions.

For a membrane that is permeable to only one type of ion, equilibrium requires that the force due to the electric field be equal and opposite to the force due to diffusion.

The equilibrium voltage associated with a given concentration ratio is expressed by Nernst equation and corresponds to the resting voltage.

Equilibrium can also be defined by equating the electrochemical potential on both sides of the membrane.

1.1.7 Active behavior of the membrane

When a stimulus current pulse is arranged to depolarize the resting membrane of a cell to or beyond the threshold voltage, then the membrane will respond with an action impulse. An example of this is seen in Figure 4 in the action potential responses 3b and 4 to the trans-threshold stimuli 3 and 4, respectively. The response is characterized by an initially rapidly rising transmembrane potential, which reaches a positive peak and then more slowly recovers to the resting voltage. This phasic behavior typifies what is meant by an action impulse.

1.2 Demyelinating diseases, effects on nerve conduction

A demyelinating disease is any disease of the nervous system in which the myelin sheath of neurons is damaged. This damage impairs the conduction of signals in the affected nerves. In turn, the reduction in conduction ability causes deficiency in sensation, movement, cognition, or other functions depending on which nerves are involved.

Some demyelinating diseases are caused by genetics, some by infectious agents, some by autoimmune reactions, and some by unknown factors.

The precise mechanism of demyelination is not clearly understood but there is good evidence that the body's own immune system is at least partially responsible.

Several central nervous system demyelinating disorders have been described in humans including multiple sclerosis.

Multiple sclerosis (MS) is a chronic, progressive, or relapsing and remitting demyelinating

disorder that affects the central nervous system (CNS) specifically and ranks as a major cause of nervous system disability in young adults.

Among the methods used to diagnose this kind of disease is the study of Motor Evoked Potentials (MEP) generated in nerve tissue after the presentation of a stimulus.
This is because in myelinated axons action potential jumps from node to node of Ranvier increasing the conduction speed through the axon.
In demyelination disorders, such as multiple sclerosis (MS), myelin sheath is affected and thereby, action potential propagation slows down, leading to deficiencies in movement, perception and cognition (17).
It is useful to use MEP technique on "Experimental Autoimmune Encephalomyelitis (EAE)" (8) as this animal model reproduces the symptoms of MS disease: inflammation, demyelination, axonal loss and gliosis.
To research the MEP in this model is useful to use TMS because the non-invasive and non-painful characteristics of this technique, thus tests can be repeated once and again on the same animals

1.3 Experimental Autoimmune Encephalomyelitis

Any technique useful to produce Motor Evoked Potentials through artificial stimulation, may be useful to study the course of the demyelinating diseases.
For instance, the primary and secondary progressive Multiple Sclerosis (MS) disease can be monitored through MEP studies and this technique may also help predict its evolution.
However, little was known about the utility of MEPs in experimental autoimmune encephalomyelitis (EAE).
EAE is preclinical model of MS with motor system involvement that can be induced in various strains of rodents.
It is an excellent model of post-vaccinal encephalitis and a useful model of many aspects of of the condition (8).

1.4 Diagnostic on nerve conduction disorders

Nervous transmission velocity, latency and signal amplitude are common measurements made during tests of nerve conduction.
Among these tests can be included the MEP generated by TMS.
Tests could be performed by magnetic stimulation of motor cortex areas and recording from

a muscle supplied by the corresponding nerve.

The time it takes for the electrical impulse to travel from the stimulation point to the recording site is recorded. This value is called the latency and is measured in milliseconds (ms). The size of the response - called the amplitude - is also measured millivolts (mV).

To get consistent and reliable results these tests require high focality induction.

1.5 Principles of Transcranial Magnetic Stimulation

According to Faraday's law, when a time-varying magnetic field is applied in the vicinity of brain tissues (or whatever object), an induced electric field appears, which in turn creates electric voltage (electromotive force) and currents useful for electrically stimulating the excitable tissue of the brain.

The magnitude of electromotive force generated is proportional to **the rate of change** of the magnetic flux $d\phi/dt$. It is also proportional to the rate of change of the current passing through the inductor coil, being the coefficient of proportionality the inductance of the coil L. The direction of emf is such that the time−varying magnetic field that results from it is opposite to that of $d\phi/dt$.

$$\mathcal{E} = -\frac{d\emptyset}{dt} = -L\frac{dI}{dt}$$ Figure 9

where L = inductance of the coil, and I = current in the coil.

That's why, not only is important the absolute magnitude of magnetic induction, but it is also essential that the waveform to be generated by the inductor coil has a **very high gradient**.

This way, magnetic stimulation is a method for stimulating excitable tissue with an electric current induced by an external time−varying magnetic field.

The non-excitable tissues are transparent for the magnetic field so that no energy losses are produced on them. Consequently, no physical damages are generated and we have that way a no painful neither invasive technique.

Those electric currents are uniformly distributed in the area of influence of the field, so that there's not any point at the scalp with potentially harmful high current densities.

These are the two main advantages of this technique over the electric stimulation: It is a non-invasive and non-painful technique, but it is still useful to selectively stimulate neuron cells.

Polson et al. reported in 1982 (7) the first successful stimulation of nerve trunks with time varying magnetic fields.

Excellent reviews of history of magnetic stimulation can be found at refs. 9 and 10.

1.6 Application areas of Magnetic Stimulation

Nerve conduction speed can be assessed by Motor Evoked Potentials (MEP) elicited by motor cortex stimulation.

Research results reveal that MEP measurements are highly reliable in a controlled environment (11).

Stimulation of motor cortex to study conduction speed can be achieved by Direct Electric Stimulation of the brain (12) or Transcranial Magnetic Stimulation (TMS) which is a painless method for motor cortex stimulation,

It is a technique that does not require surgery or deep anesthesia. Magnetic stimulation can be applied to nervous stimulation either centrally or peripherally.

The main benefit of magnetic stimulation is that the stimulating current density is not concentrated at the skin, as in electric stimulation, but is more equally distributed within the tissue. This is true especially in transcranial magnetic stimulation of the brain, where the high electric resistivity of the skull does not have any effect on the distribution of the stimulating current. Therefore, magnetic stimulation does not produce painful sensations at the skin, unlike stimulation of the motor cortex with electrodes on the scalp.

Another benefit of the magnetic stimulation method is that the stimulator does not have direct skin contact. This can be a benefit if you have to work in a sterile operation environment.

Available commercial products

As mentioned, the first papers introducing the clinical application of magnetic stimulation were published in 1985. Now magnetic stimulation devices for clinical applications are produced by several manufacturers, among most importants are: Magstim, Magventure, Cadwell, Brainsway and Neuronetics (Fig.11).

Magventure MagPro R100 circular coil

Magstim 8-shaped double coil

Fig 11: Some available commercial products of TMS

The strength and getting into focus capacity (focality) of induced electric field has been comprehensively studied for every type of coil design. The results for 50 different coil designs have been recorded (13).
Among those TMS coil designs, it was seen that for most applications, a trade-off between electric field **depth of penetration and focality** is necessary.
In general it can be said that 8-shaped coils are more focal than circular ones. For any coil design, the ability to directly stimulate deeper brain structures is obtained at the expense of inducing wider electrical field spread.
There have been done some study of commercial TMS on rats and, not surprisingly, poor focality of stimulation has been reported (14).
This can be a problem when some application as somatotopic mapping or corticospinal pathway health conditions, are being researched.

The need to have equipments specially adapted to mouse motor cortex
Most of available products are designed for human therapy so that its dimensions, magnetic field strength, focality, etc, are no suitable to research on mouse.
Taking into account these data and the final objective of our project, which is to stimulate the superficial motor cortex of mouse in order to research demyelination of axons, we have chosen a design that maximizes the focality even at expense of depth of penetration.
The chosen shape has been a solenoid with ferrite core of Ø 6 mm.

1.7 Effects on nerve excitability

The effects of stimulator design on nerve excitability rely on many factors:

1. Coil dimensions
2. Inductor heads arrangement (Shape)
3. Air/Ferromagnetic core
4. Focality of induced electric field
5. Magnetic field strength and gradient
6. Pulse duration and polarity
7. Locus of activation
8. Waveform of inductor voltage

9. Direction, orientation, strength and gradient of induced electric field
10. Topographic location, as well as electric permittivity and magnetic permeability of neuronal tissue to be stimulated

All these factors have to be taken into account to design the stimulator device suitable for a given application.

Is not yet exactly known the magnetic induction strength, neither the depolarization induced, necessary to trigger an action potential at each neuron of EAE mouse.

That's why, at the time of designing the prototype, we had no enough data to look for a final prototype and additional studies are necessary to assess the influence of some of them.

2 Objectives

Taking into account the mentioned effects of parameters on nerve excitability and that we have not at this time a mathematical model of induced electric field in mouse brain, the work's objective at this moment has not been to have a device able to elicit an action potential but just:

1. **To design and build a "scale prototype" of TMS** specifically designed to stimulate **small areas** ($\leq$30 mm^2) of the mouse motor cortex in a spatially focused way.
   The prototype should operate with reliability in a repetitive way.
2. A high capacity to be **focused on small area** should be preferably, even at expense of a smaller depth of penetration.
3. To concentrate the magnetic flux lines as much as possible a **ferrite/soft iron core** has been chosen.
4. The prototype should be able to generate a waveform with a **rising time shorter** than **100 µS** and a duration **≤ 10 mS** .
5. With regard to magnetic field strength and gradient, an **easily scalable** design was chosen.
6. An stimulation device easy to apply in **any stimulation locus** with **selectable direction and orientation**.
7. **To assess its capacity** to : stimulate in a noninvasive way, the neuronal connections of the mouse brain through the application of single or repeated magnetic pulses

In a second phase, through mathematical modelling and COMSOL software, it will be developed a simulation of the mouse's brain in order to predict the

- **Induced Electric field** (in different structures of the motor cortex for a given magnetic field)
- **Depolarization voltage** we should generate, and
- **Activation threshold voltage**

That way, we will be able to anticipate the magnetic stimulation necessary to get a depolarization voltage higher than threshold.

3 Materials and Methods

3.1 Scale made Prototype: design and construction

It is known that the stimulation threshold for **different types of neurons** depends, among other factors, on the pulse **waveform** and **current direction** (15, 16).
Therefore, for our design the next aspects have been considered:

1. Types of neurons, its situation and specific points of stimulation:
    a. Fiber bends for pyramidal tract neurons,
    b. Axonal tracts of cortical and relay neurons
    c. A combination of both for pyramidal association fibers

2. Coil shape, and materials it is made of.

3. Electromagnetic interrelated parameters of TMS equipment.
   The electronic circuit that has been designed is:
    a. Modular and Portable
    b. Battery powered
    c. Adjustable range of magnetic induction
    d. Adjustable range of derivative of magnetic induction with respect to time (changing R and L; C in the future)
    e. Able to be synchronized through thyristor gate pulse control
    f. Hand/Automatic triggered
    g. Magnetic flux highly focusable on target areas

4. The purpose of this work has been to build an actual prototype of electronic circuit and magnetic inductor device, able to generate and apply such magnetic flux

density highly focusable on neuronal area of interest.

5. Waveform of magnetic pulse and stimulation current, the critical elements that have been considered are:
    a. Actual value of Magnetic Induction (B)
    b. Maximum rate of magnetic induction in the coil (dB/dt) and, consequently, of electric voltage and intensity in the coil (dV/dt, dI/dt).
    c. Stimulation pulse's duration
    d. Monophasic pulse

6. Stimulation electric field threshold (Volts/mm) for any given target neuron's area.

7. Orientation of magnetic pulse and induced electric field; Direction of the induced current in the brain (di/dx, being x the predominant axon's group direction).

8. Mechanisms and loci of neuronal excitation.

9. Calculation of magnetic flux passing through any given surface.

10. Calculation of magnetic induction in any given surface.

3.2 Scale made Prototype's components and systems

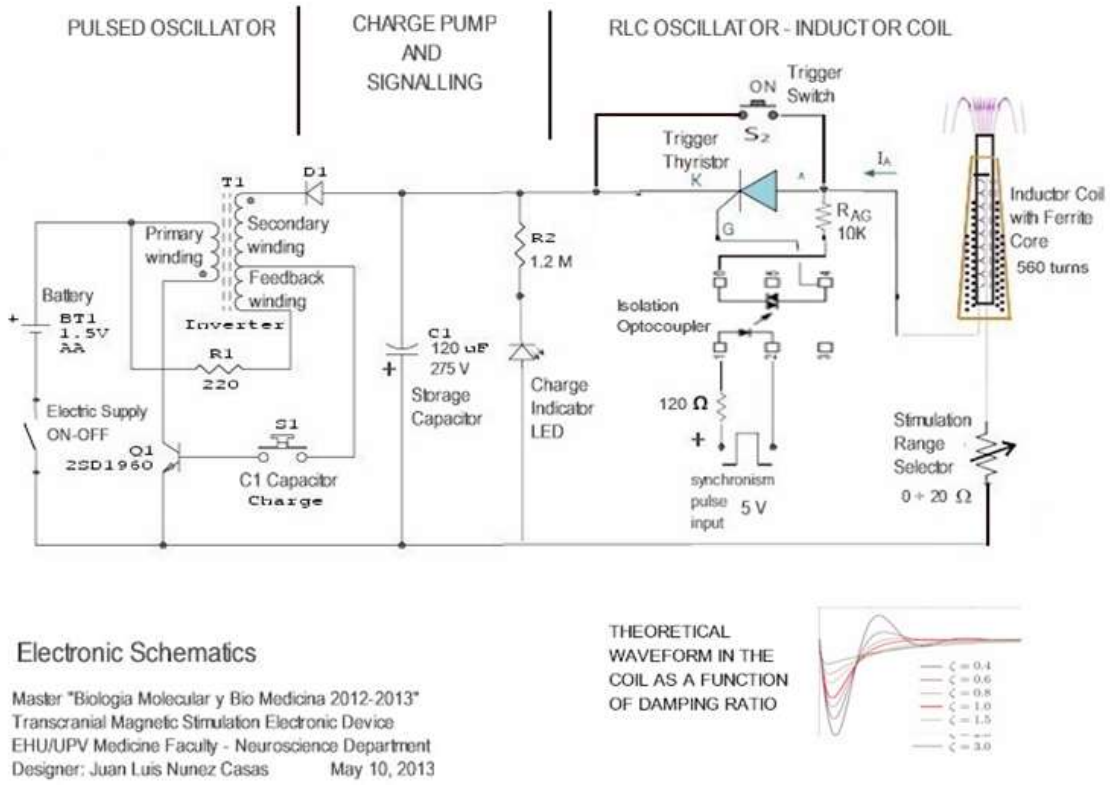
The scale prototype built has two main parts: Electronic Pulse Generator and Inductor Coil.

The electronic project has 6 separate building blocks:

1. Pulsed oscillator
2. Charge-pump and Signalling
3. Discharge circuit (RLC Oscillator-Inductor Coil)
4. Magnetic Inductor Coil

3.2.1 Electronic Schematics

Fig 11: Electronic Schematics

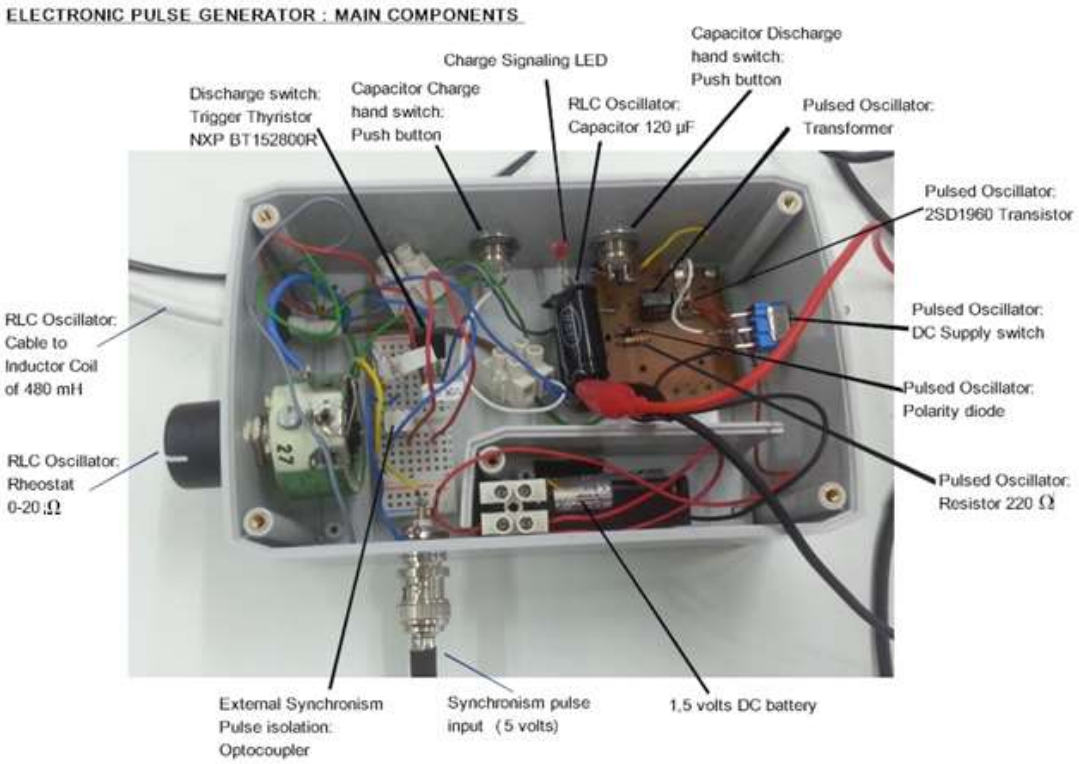


The electronic pulse generator which has been designed, is based in a RLC oscillator with power controlled through the thyristor and rheostat.

An optocoupler has been used to drive the thyristor from an external control pulse generator and to synchronize with the data acquisition system

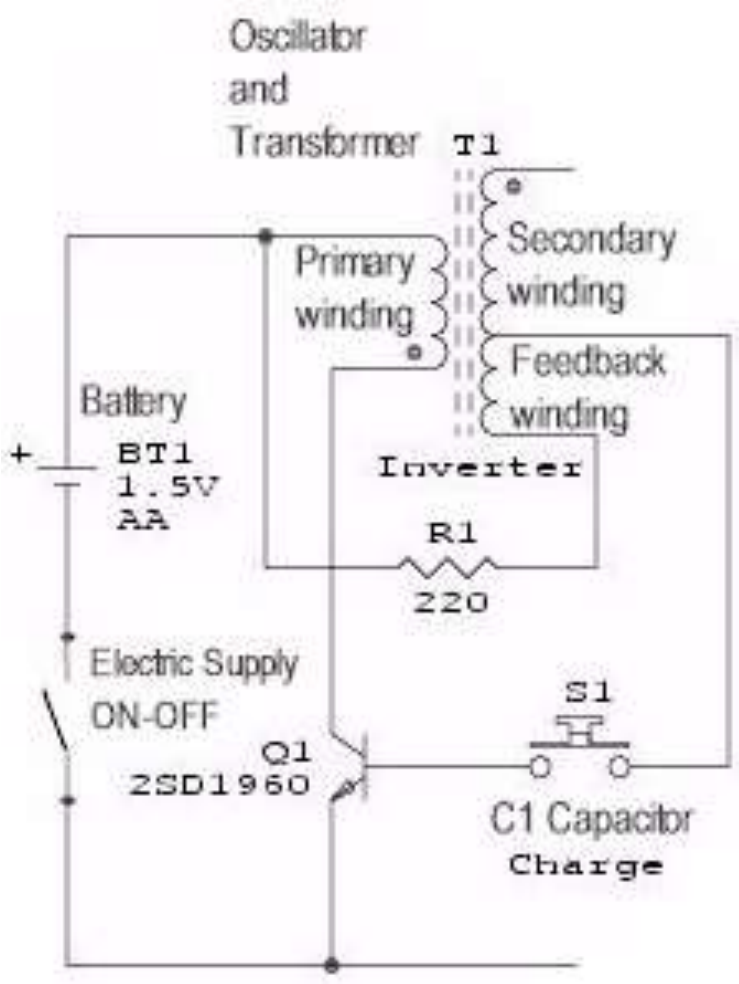
3.2.2 Actual scale prototype of electronic pulse generator

Fig 12: Electronic pulse generator: main components



3.2.3 The Pulsed Oscillator

Fig. 13**:** The pulsed Oscillator



It consists of next components:

- Electric supply (switch and battery of 1,5 Volts DC)
- Transformer-Inverter
- Transistor
- Resistor
- Charge manual switch

When the charge switch S1 is hand closed an electric current starts flowing into the transistor base.

The base becomes activated turning on the transistor, and a small current flows through

the feedback winding of transformer.

According to the Faraday's law, the magnetic flux produced by this current induces a voltage in the primary winding and a current starts flowing through the collector of transistor.

The magnetic flux expands and grows getting stronger until it reaches a maximum.

At that point, the flux is stationary and a stationary flux doesn't produce a voltage or current in the other windings.

That way, the flux in the feedback and secondary windings starts to collapse, also the current to reduce.

An opposite (inverted) electromotive force is generated in the winding, trying to maintain **the** previous magnetic flux.

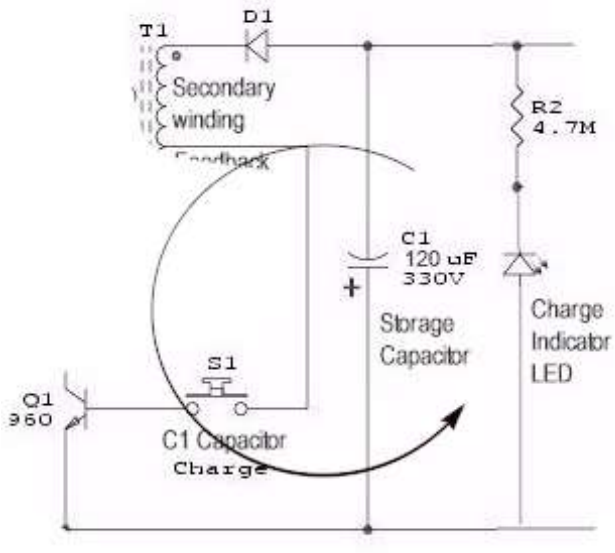
Thus the output voltage from secondary winding is reversed and reaches a high value before collapsing.

Because of this, the current in the feedback winding starts to reduce and the transistor to turn off.

When the transistor is fully off, the reverse voltage from the feedback winding ceases and thanks to the battery supply, the transistor turns on again repeating the cycle.

3.2.4 Charge-Pump and Signaling

Fig. 14: Charge-Pump and Signaling

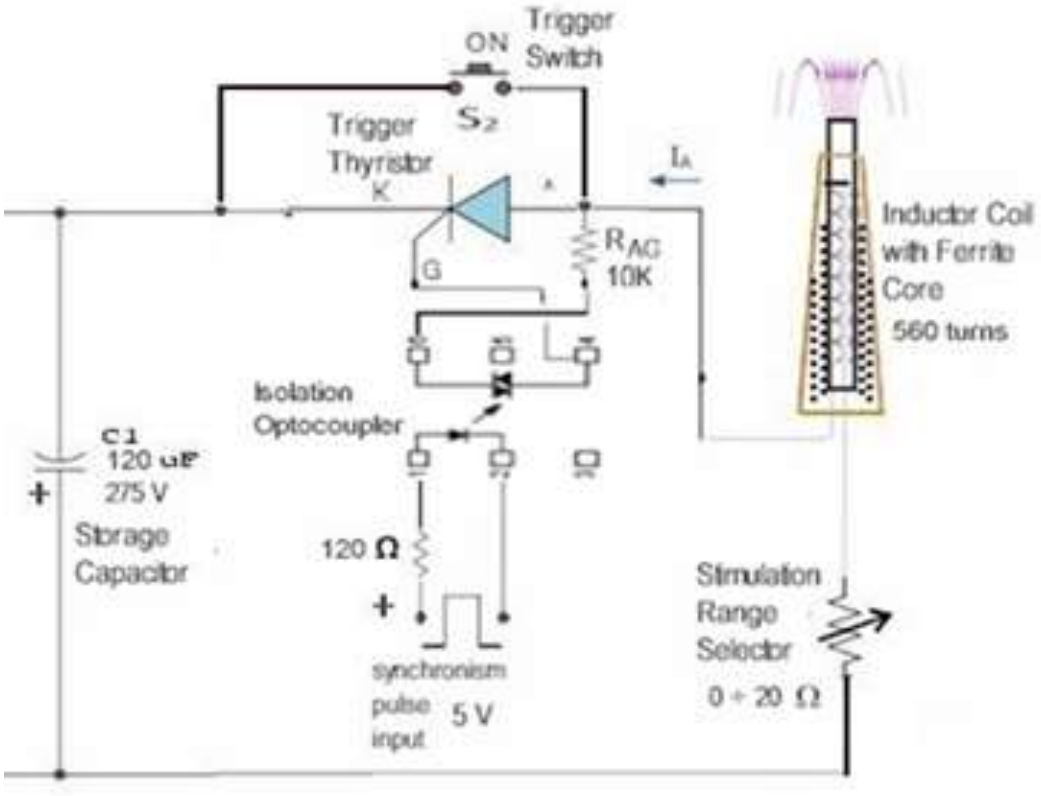


The output of voltage from the secondary of transformer is a sinewave with an amplitude of ≈ 300 Volts.
This voltage creates a current that charges the Storage Capacitor C1 and that is fed into the high-speed diode D1. This diode only allows the negative pulses of current to pass through, from the Anode to the Cathode (towards the transformer).

When the charge reaches the specified voltage, the LED lights signaling this state.
When the charge finishes, the switch S1 is released and only the right part of the circuit matters ahead for the operation.

3.2.5 Discharge Circuit

Fig 15: Discharge Circuit



The magnetic discharge system is a classic RLC (resistor-inductance-capacitor) oscillator circuit.
This circuit exhibits oscillatory behavior when it is disturbed from their position of static equilibrium.

In Fig. 11 are recorded the theoretical waveforms that can exist for different damping ratios (Fig 16), showing underdamped and overdamped responses of the RLC circuit.

$$\zeta = \frac{R}{2}\sqrt{\frac{C}{L}}$$

Fig. 16 Damping ratio for a RLC oscillator circuit

3.2.6 Actual prototype of Magnetic Inductor Coil

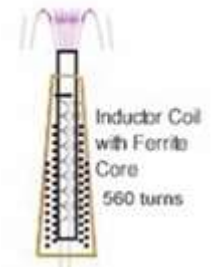


Fig 17: Actual prototype of Magnetic Inductor Coil (Solenoid)

Hand made winding coil

A ferrite core of Ø=6 mm has been chosen, with a relative magnetic permeability μ_r = 640. Around this core has been wrapped a copper wire of Ø= 0,6 mm, with 560 turns in 4 layers, resulting an inductance L≈ 480 mH and a final diameter of Ø = 17 mm.

3.2.7 Actual test layout

Fig 18: Actual test layout

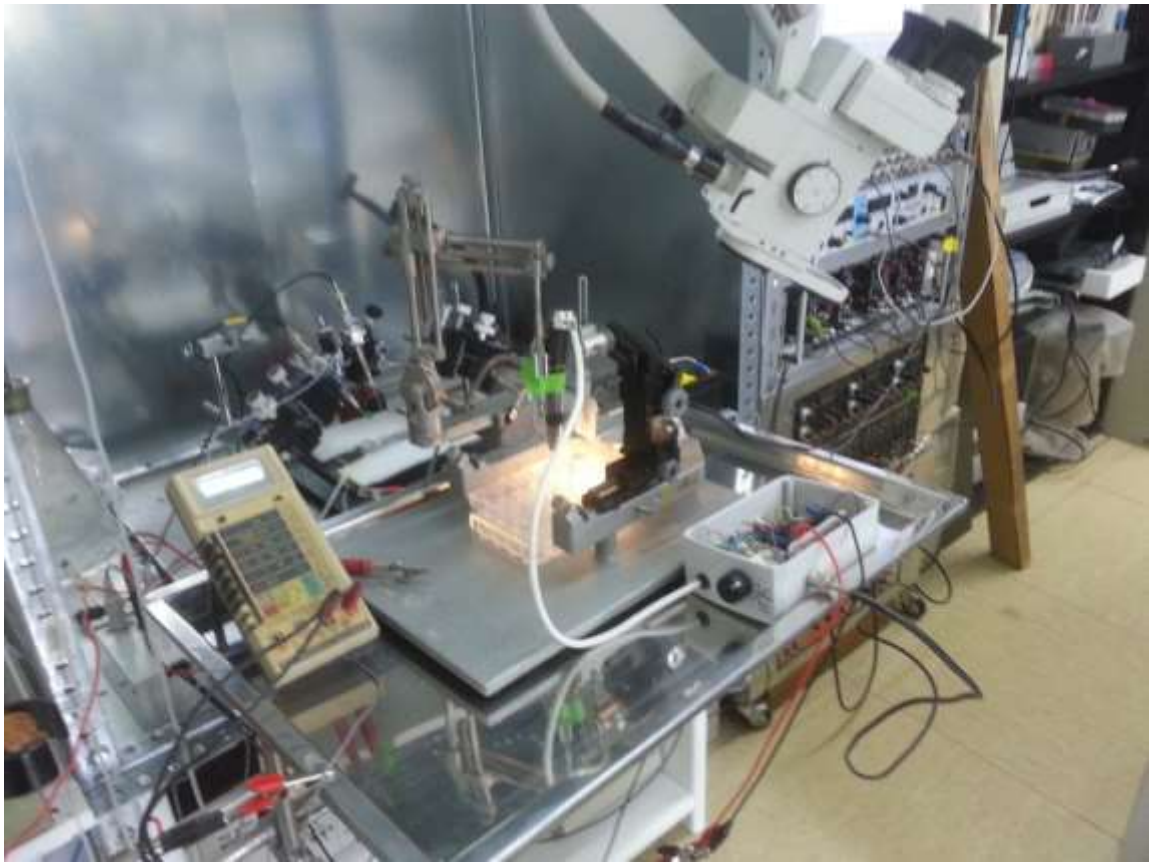


Figure 18. Test layout

3.3 Data collection: In vivo tests

One adult BALB/c mouse was used to compare electric and magnetic stimulations. The animal was anesthetized (*Avertin* (2, 2, 2-Tribromoethanol).

Recording of motor evoked potentials from relaxed muscle require sedation of the animal. The applied anesthetic product and its concentration can also influence the elicitation of Motor Evoked Potentials or its abolition (14), Avertin instead doesn't abolish MEPs. Electric stimulation assessed by a bipolar silver electrode placed over the motor cortex connected to a WPI pulse generator and WPI stimulus isolator (70 V constant voltage, 0.2 Hz).

Electromyographic (EMG) recording was done with an concentric electrode (Medelec Type 21001) placed in the rear limb. Signal was amplified (5000X) (Neurolog System) and digitized (interval 3μs) (DigiData 1200A and Axoscope 8, Axon instruments). TMS stimulation at 0.2 Hz.(Fig. 4).

4 Results

4.1 Voltage in solenoid terminals ,Waveform in oscilloscope

Voltage and current overdamped waveform (Fig 19) were measured in terminals of solenoid coil ($V_p \approx 320$ V, $I_p \approx 15 \div 160$ A , Voltage gradient ≈ 7 V/μs) and recorded waveform in the Oscilloscope below.

Figure 19. Voltage Waveform and gradient:
Rise time ≈ 50 μS ; Decay time ≈ 5 mS

4.2 Magnetic field measurement

The magnetic Induction produced close to the coil tip was ≈ 0,4 $T_{max.}$

It was measured with a Gaussmeter Hirst Model GM07 kindly provided by Dr. Jon Gutierrez (Sciences Fac.UPV-EHU) for this test.

4.3 Measurement taken working on animal : waveform, latency

Figures 20 and 21: Electromyographic (EMG) records in the muscle.

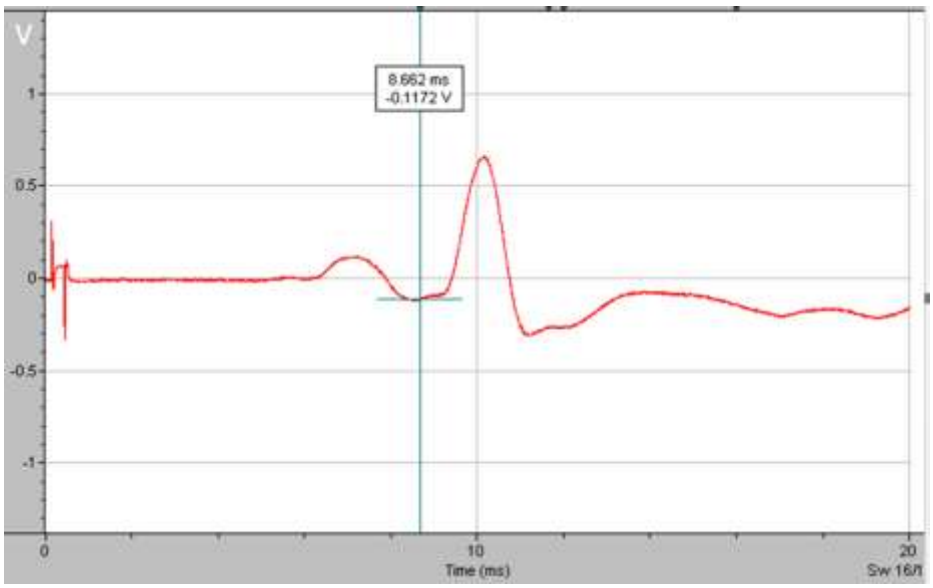


Figure 20. Direct Current Electric Stimulation in Motor Cortex of mouse

This MEP Fig20, got with Direct Electrical Stimulation is included as comparison with TMS results in Fig21.

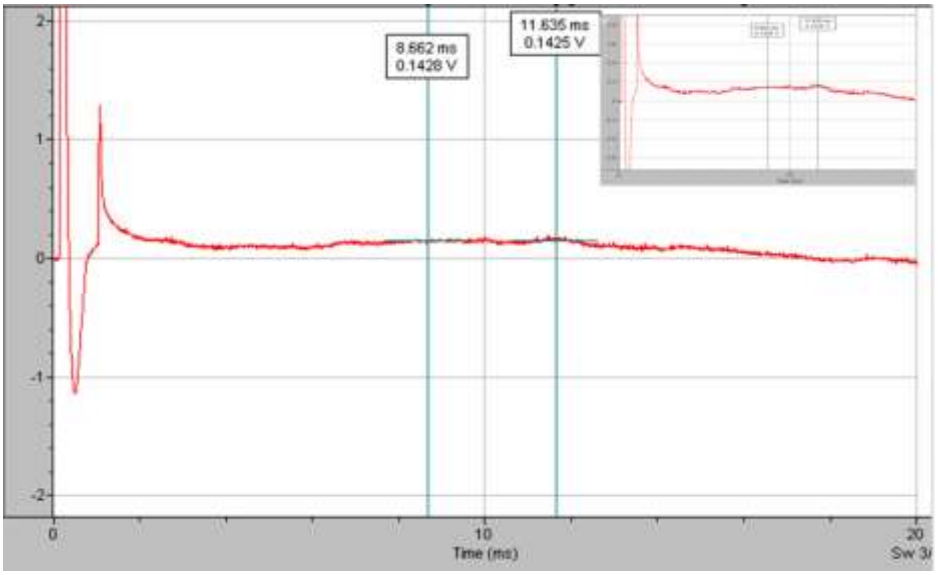


Fig 21: Transcranial Magnetic Stimulation in Motor Cortex of mouse

5 Discussion

As previously mentioned, Our TMS elicited was enough to see a light increase of the EMG activity with the same latency as electric stimulation (~10 ms).
Even if we have built a **small scale prototype**, some electromyographic activity can be recorded in the muscle for the same latency (~10mS) in which a MEP was recorded during the Direct Electric Stimulation test.
That part of the graph should be totally flat as it records the electromyographic activity in the muscle which is resting.
Anyway, this cannot be considered as a final validation of our prototype because we didn't actually get the typical waveform of a MEP.

This work has been exploratory to gain experience and to verify basic details of design.
Its validity has to be based in the working parameters we have been able to generate such as:

- Voltage at coil terminals
- Gradient of voltage
- Waveform
- Repetitive operation and
- Rest of achieved parameters listed in point 2.

The scaling up of these parameters should be easy thanks to the design used and the experience gained.

6 Conclusions and Further research

1. The **waveform and gradient of voltage were suitable** for TMS according to the parameters usually found at industry (Ref. 1).

2. **A scale prototype** of TMS **specifically designed for mouse** brain was **successfully built** according to objectives (The electrical voltage supply has been **just 1,5 volts** which is considerably low when compared to commercial products that tend to have hundreds of volts).

The maximum Magnetic Induction we have got has been 0,4 Teslas, which is no much smaller than the 1,5-2 Teslas usually found in commercial products.
No motor evoked potential was elicited in our TMS test, but based in the engineering parameters that have been measured, and the observed waveform, we consider the scale prototype as suitable to stimulate directly small areas of mouse brain thanks to the capacity of ferrite core to concentrate magnetic flux lines in smaller areas.

To get an actual MEP it should be previously **necessary to scale up the device** until higher discharge voltages were obtained.
It should be also necessary to refine some other characteristics as for example:
a smaller area of ferrite core tip, to apply biphasic/polyphasic voltage wave and to adjust RLC parameters as necessary to get a sub-damped waveform.
Once these steps are done, it should also be necessary to stimulate the mouse brain in several direction, orientations and positions according to the higher sensitivities predicted in a simulated model of brain's excitable area.

3. Further research:

- Simulation of the morphology of electric field induced in mouse brain by a variable magnetic field, using COMSOL software.
- Determination of threshold voltage able to trigger MEP in simulated motor cortex of mouse.
- Scaling up the current device to reach that stimulation threshold, e.g. rising voltage, shaping the tip of ferrite core as a truncated cone, adjusting RLC parameters to get a sub-damped waveform, using inductor core of soft iron instead ferrite, and biphasic magnetic pulse.

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