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

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Introduction

The myelin sheath is a membrane spirally wrapped around the axon formed by proccesses of olygodendrocytes (in Central Nervous System) 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.

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.

"Experimental Autoimmune Encephalomyelitis" is an animal model used for studying demyelination which reproduces the symptomatology of MS. (C.S. Constantinescu et al., British Journal of Pharmacology (2011) **164** 1079–1106)

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 (R. Lefebvre et al.,J Manipulative Physiol Ther. 2004 Feb;27(2):97-102.).

Stimulation of motor cortex to study conduction speed can be achieved by direct electric stimulation of the brain (Matute et al., J Neurosci. 2007 Aug 29;27(35):9525-33) or Transcranial Magnetic Stimulation (TMS), (Muscle Nerve. 2006 Feb;33(2):265-73).

TMS (Fig.1) is a painless method for motor cortex stimulation which does not require surgery or deep anesthesia (Alan Cowey-Phil. Trans. R. Soc. B 2005 360, doi: 10.1098/rstb.2005.1658, 29 June 2005, p1187).

Figure 1. Some available commercial products of TMS:
(A) Magstim 8-shaped double coil
(B) Magstim model 200 Circular coil

Using a small solenoid with ferrite core allows the magnetic field to be focused in a small brain volume and, consequently to use lower magnetomotive force inductors compared with other devices used in rats (e.g. Luft et al. Exp. Brain Res. 2001, 14=:112-21; Rotenberg et al., 2009, Clinical Neurophusiol. 121: 104-8).

Objectives

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 the work's objective at this moment has not been to elicit an action potential but just :

- **to design and build a scale prototype of TMS,** specifically designed to stimulate small areas of mouse motor cortex.
- **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

Figure 2. Scale Prototype: (C) Solenoid coil built

Materials and Methods

The stimulation threshold for different types of neurons depends, among other factors, on the induced field strength and direction and the pulse waveform (Salvador, et al. Clin Neurophysiol. 2011 April; 122(4): 748–758).

Therefore, for our design of stimulation device, have been considered the next variables:

- Magnetic field of variable strength
- Variable pulse waveform
- Selectable direction and orientation of induced electric field
- Able to be focused on small and specific areas of mouse's brain

For those reasons, a solenoid with ferrite core and small diameter (17 mm, Fig 2) was chosen for inductor coil. It has been hand coiled over a ferrite core of Ø 6mm, a solenoid of 560 turns with an inductance of ≈ 480 mH.

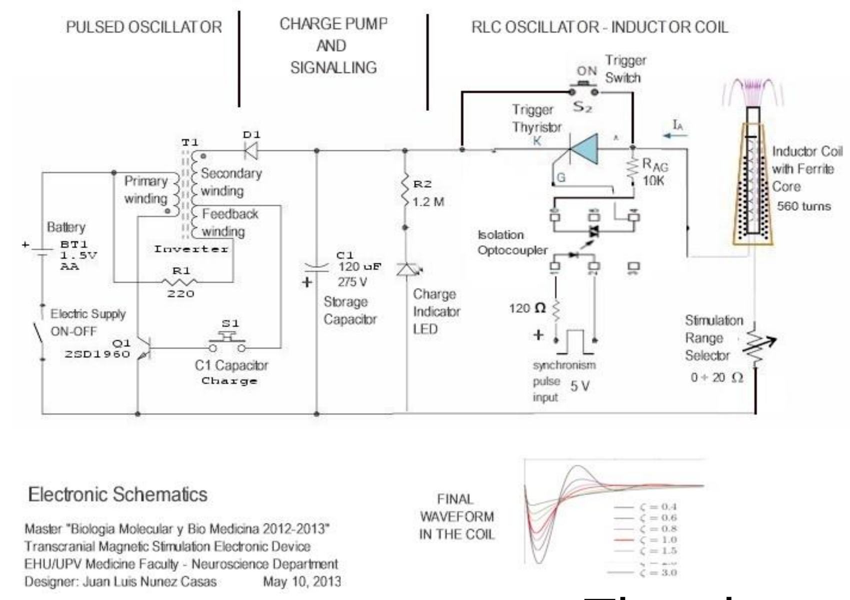


Figure 3. Electronic schematics of pulse generator

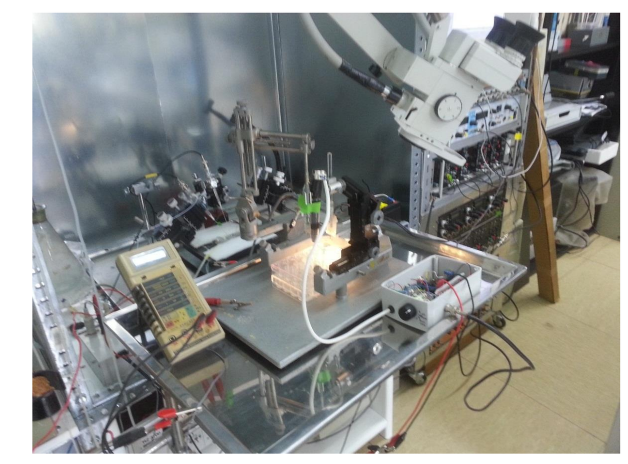


Figure 4. TMS test layout

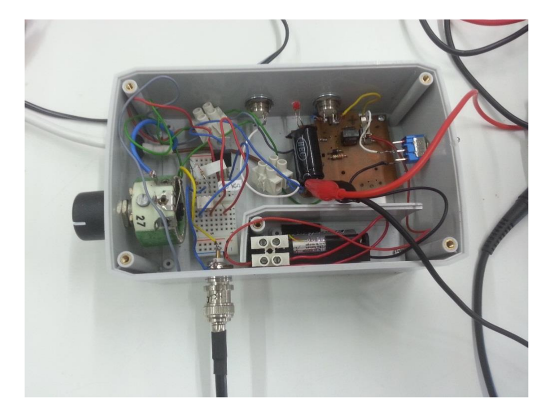


Figure 5. Detail of the prototype TMS electronic component and circuits

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 (Figs. 3 and 5).

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

Results and Discussion

•Voltage and current overdamped waveforms (Fig 6) were obtained in terminals of solenoid coil (V_p≈320 V, I_p≈15÷160 A , Voltage gradient ≈ 7 V/μs).

•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.

Figure 6. Voltage Waveform and gradient: Rise time ≈ 50 μS Decay time ≈ 5 mS

The magnitude and gradient of magnetic field produced by the device can be adjusted through variable resistance and inductance.

Our TMS elicited was enough to see a light increase of the EMG activity but with the same latency as electric stimulation (~10 ms).

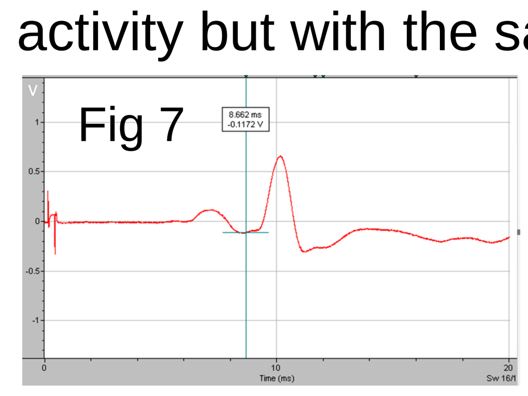
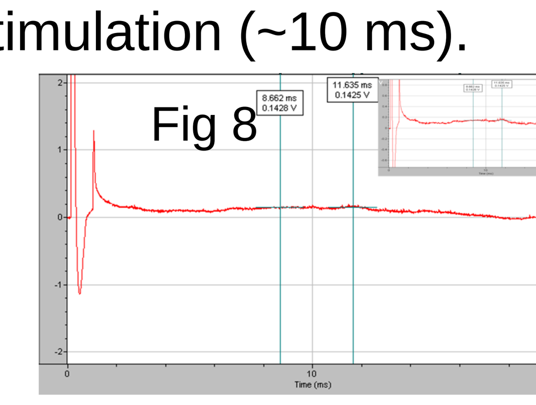


Fig 7: Motor cortex direct electric stimulation
Fig 8: Motor cortex TMS



Conclusions

1. A scale prototype of TMS specifically designed for mouse brain was successfully built according to objectives.
   It was suitable to stimulate directly small areas of mouse brain thanks to the capacity of ferrite core to concentrate magnetic flux lines.
2. The waveform and gradient of voltage were suitable for TMS according to the parameters usually found at industry (Alan Cowey-*Phil. Trans. R. Soc. B* 2005 360, doi: 10.1098/rstb.2005.1658, 29 June 2005, p1188).

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 evoked potentials 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.