Universitat Autònoma de Barcelona

THE TMSTROKE STUDY. TRANSCRANIAL MAGNETIC STIMULATION IN ACUTE ISCHEMIC STROKE: MOTOR PROGNOSIS AND NEUROPHYSIOLOGICAL FEATURES

GIUSEPPE LUCENTE

PHD PROGRAMME IN MEDICINE

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PhD Thesis

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"I have no special talent. I am only passionately curious."
"The important thing is not to stop questioning."

Albert Einstein

“What we know is a drop, what we don't know is an ocean.”

Isaac Newton

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And although you won't be probably be born yet, I want to thank my baby, for all the kicks and punches you gave me while you were floating in mum's belly. You listened to all this already many many times..

List of abbreviations

TMS: Transcranial Magnetic Stimulation

Ms: milliseconds

mA : milliampere

T: tesla

MEP: motor evoked potential

CMAP: compound muscle action potential

D wave: Direct Wave

I-Wave: indirect Wave

cSP: cortical silent period

CMCT: central motor conduction time

PMCT: peripheral motor conduction time

M1: primary motor cortex

S1: primary sensory area

CST: corticospinal tract

PM: premotor cortex

SMA: supplementary motor area

rMT: resting motor threshold

ADM: abductor digiti minimi

FDI: first dorsal interosseus

αMNs: alpha moto neuron

HUGTIP: Hospital Universitario Germans Trias I Pujol

mRS: modified Rankin Scale

NIHSS: National Institute of Health Stroke Scale

BMC: British Medical Council

MSO: maximum stimulator output

COPD: chronic Obstructive Pulmonary Disease

TICA: Terminal Internal Carotid Artery

MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery

ICA Internal carotid artery;

tPA: Tissue Plasminogen activator

EVT: Endovascular Treatment

TOAST: Trial of ORG 10172 in acute stroke treatment

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ABSTRACT

Introduction and aims

Stroke is the leading cause for disability in the western world. Transcranial magnetic stimulation is a versatile instrument, which allows to study the physiology and pathophysiology of human brain non-invasively. The aim of this study is to explore the correlation between neurophysiological features in acute stroke patients measured at different time points and the disability at 3 months, both regarding the motor and the sensory pathways.

Methods

We enrolled 78 acute ischemic stroke patients (64.5±9.4 yo; 62.8% Males). A battery of neurophysiological motor (rMT, MEP, cSP , CMCT) and sensory tests (sensory and pain thresholds) were performed, as well as extensive clinical evaluation at admission, day 7 or discharge correlating the data with the 3-monts outcome measured by functional disability scale (mRS)

Results

MEP features and sensory impairment correlated strongly with functional outcome at 3-months follow-up. The presence of the MEP by itself was associated to a good outcome (90.9% vs 13.6% p <0.001) both in the proximal and distal muscle studied. Cortical excitability was related to a better outcome, as well as sensory impairment. An improvement of MEP amplitude of 0.5mV (AUC 0.916; Sensitivity 74%, Specificity 100%) was independently associated with a good prognosis at 3-months (mRS ≥ 2).

Conclusions

Acute stroke provokes profound changes in the cortical function and pyramidal tract. Those changes are strictly related to functional outcome at 3-month. An increase of 0.5mV between basal and day 7 studies is related to a better functional outcome at 3months, and the degree of sensory impairment is related to the functional outcome as well.

RESUMEN

Introducción y objetivos

El ictus es la principal causa de discapacidad en el mundo occidental. La estimulación magnética transcraneal es un instrumento versátil que permite estudiar la fisiología y fisiopatología del cerebro humano de forma no invasiva. El objetivo de este estudio es evaluar la correlación entre las características neurofisiológicas en pacientes con ictus agudo medidas en diferentes momentos y la discapacidad a los 3 meses, tanto en las vías motoras como sensitivas.

Métodos

Se incluyeron 78 pacientes con accidente cerebrovascular isquémico agudo (64,5±9,4 años; 62,8% hombres). Se realizó una batería de pruebas motoras neurofisiológicas (rMT, MEP, cSP, CMCT) y sensitivas (umbrales sensitivos y de dolor), así como una evaluación clínica extensa al ingreso y posteriormente el día 7 o al alta, correlacionando los datos con el estado funcional a los 3 meses según la escala de discapacidad funcional (mRS).

Resultados

Las características de MEP y el estado de las vías sensitivas se correlacionaron con el resultado funcional a los 3 meses de seguimiento. La presencia del MEP tanto en músculo proximal como en distal se asoció independientemente a una menor discapacidad (90,9% vs 13,6% p<0,0001). En particular una mejora de la amplitud de MEP de 0,5 mV (AUC 0,916 sensibilidad 74 %, especificidad 100 %) entre el estudio basal y a los 7 días se asoció con un buen pronóstico a los 3 meses. La excitabilidad cortical y una menor alteración sensitiva se relacionaron también con una mejor recuperación.

Conclusiones

El ictus provoca cambios significativos en la función cortical y el tracto piramidal. Esos cambios se encuentran estrictamente relacionados con el resultado funcional a los 3 meses. Un aumento de 0,5 mV en MEP entre las exploraciones basales y las del día 7 se relaciona con un mejor resultado funcional a los 3 meses, de la misma forma que un mejor rendimiento sensitivo y de excitabilidad cortical.

1. INTRODUCTION

The history of transcranial magnetic stimulation (TMS) begins more than 150 years ago, with Michael Faraday's crucial discovery of the electromagnetic induction. Faraday discovered that an electrical current flowing in a copper wire produces a magnetic field with a direction perpendicular to the former. At the time of this discovery, it was already known from Luigi Galvani's (1737–1798) experiments that nervous tissue responded to electricity. Galvani had found that frogs' legs twitch when the muscles are placed against two different metal conductors. He concluded that these findings proved that frog legs and indeed all muscles have internal electricity .

1.1 History and development of Transcraneal magnetic stimulation

The first magnetic field induction application in neuroscience comes from France, in 1896. The French physician Arsène d'Arsonval reported the induction of phosphenes by magnetic field. He build a so called "Apparatus for Measuring Alternating Currents of All Frequencies" capable of delivering an alternating magnetic field of 110 volts, 30 amperes and a frequency of 42 cycles per second. Once turned on, when one placed the head into the coil it provoked phosphenes and vertigo, and in some persons loss of consciousness.

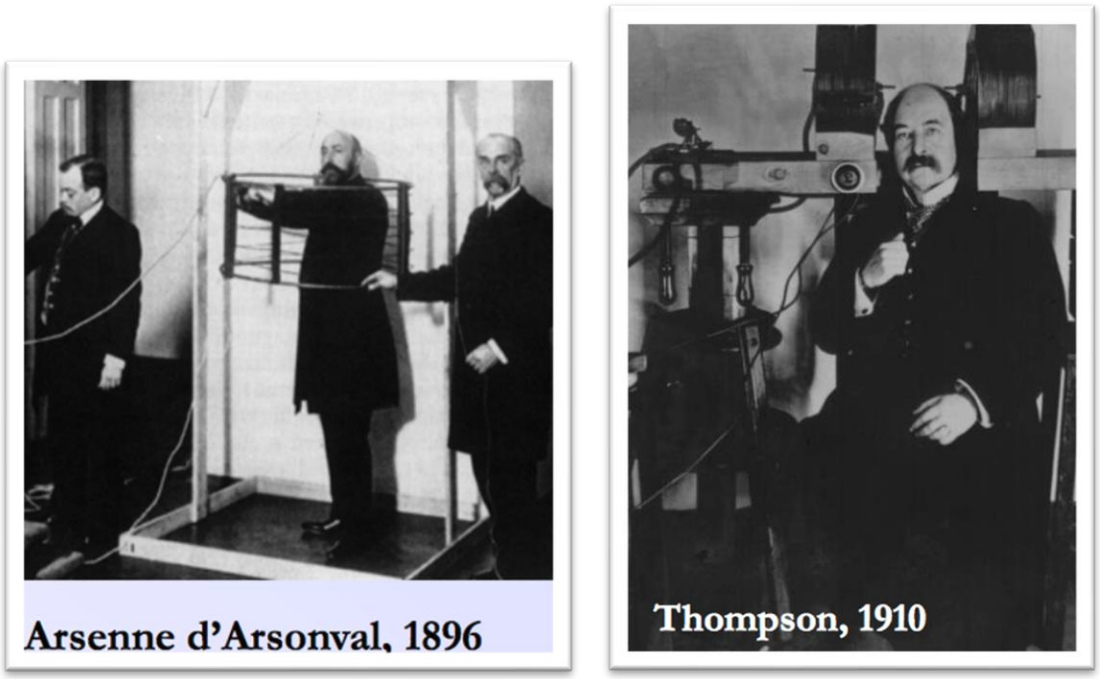

Figure1. *Arsenne d'Arsoval and Thompson's first magnetic stimulation equipment*

In the twentieth century, as alternating current began to replace direct current as a source of electrical energy, it became easier for experimentalists to generate alternating magnetic fields. The scientist embarking the study of the effects of magnetism on human brain was Silvanus Philips Thompson. He constructed a large 32-turn coil (23 cm diameter and 20cm long) in 1910 and applied up to 180 amperes of power-line current to it, generating a peak maximum intensity at the center of the coil of approximately 1,400 Gauss. He could generate what himself called "magnetophospene", the sensation of illumination, flickering that could be perceived with the eyes closed or open, in the dark or in daylight. Furthermore, several of his subjects also noticed a strange taste after two to three minutes of exposure to Thompson's apparatus, a perception currently inducible by TMS and probably related to transcranial activation of the facial nerve and the corda tympani.

The actual first placebo controlled experiment was developed by Knight Dunlap(1) at Johns Hopkins University. He believed that the findings reported were due to the loudness of the sound produced by the current flow through the transformer. In 1911, in an attempt to design a cleaner experiment, Dunlap constructed a 27-turn elliptical coil (20m cm high and 35cm in diameter), which was suspended from the ceiling and could be lowered over the subject's head. Subjects wore earplugs and when current was not flowing through the head coil, it was delivered to a resistor that caused the transformer to produce the same sound as when the current flowed through the head coil. Dunlap tested with 200 amperes of current at 60 Hz, some subjects experienced flickering phosphenes, but others did not. In 1911, Magnusson and Stevens constructed two coils with elliptical cross sections. These coils could be used singly or arranged coaxially, and direct or alternating current was passed through the coils surrounding subjects' heads. No sensation was perceived when the direct current was flowing, but sensations were experienced when the direct-current flow started or arrested. When the direct current was initiated, subjects perceived a luminous horizontal bar moving downward. When the direct current was arrested, the luminous bar moved upward. When alternating current was applied flickering lights appeared and were brightest at a current frequency of 20–30 Hz. They then tried to determine at what point in the visual pathway magnetic stimulation was actually acting. The scientists pursued this question by attempting to stimulate nerves outside the visual system using a sciatic nerve of a cat. They carried out an unsuccessful experiment with a special coil that applied a 60 Hz alternating current to the exposed sciatic nerve of a cat.

Since then, very little interest was dedicated to magnetic stimulation until the mid 40s. In 1946, Walsh(2) reported the induction of phosphenes using an iron-core coil placed

adjacent to the eye. Walsh's findings were reproduced by Barlow (3)and colleagues (1947), who constructed a small coil surrounding a laminated iron core. The coil was placed adjacent to one temple. Alternating current of 10 to 40 Hz was applied, producing both colorless and colored flickering-light sensations. He also found that no phosphenes were perceived when the coil was placed over the occipital bone. On the basis of this evidence, he concluded that magnetophosphenes were generated through stimulation of the retina and not in the visual pathways or the visual cortex. Several other investigators went on further to characterize the nature of magnetophosphenes (4–6), including Lovsund et al(7). , who performed a quantitative analysis of threshold values for the generation of magnetophosphenes and also confirmed Barlow and colleagues' earlier claims that these sensations originated in the retina.

In 1959, Alexander Kolin and colleagues(8), constructed an excitatory coil surrounding a bar electromagnet with a pyramidal pole tip. They showed for the first time, that an alternating magnetic field could stimulate nervous tissue in vitro, isolating a frog sciatic-nerve-gastrocnemius-muscle preparation and looped the sciatic nerve around the pole of the magnet. Contraction of the gastrocnemius muscle was obtained when both 60 and 1,000 Hz were applied to the coil. This experiment offered the first definitive proof that a magnetic field could induce enough current to stimulate a motor nerve.

The very first human brain magnetic stimulation was held on 12 February 1985 by the Sheffield group with a more powerful and efficient magnetic stimulator in London.

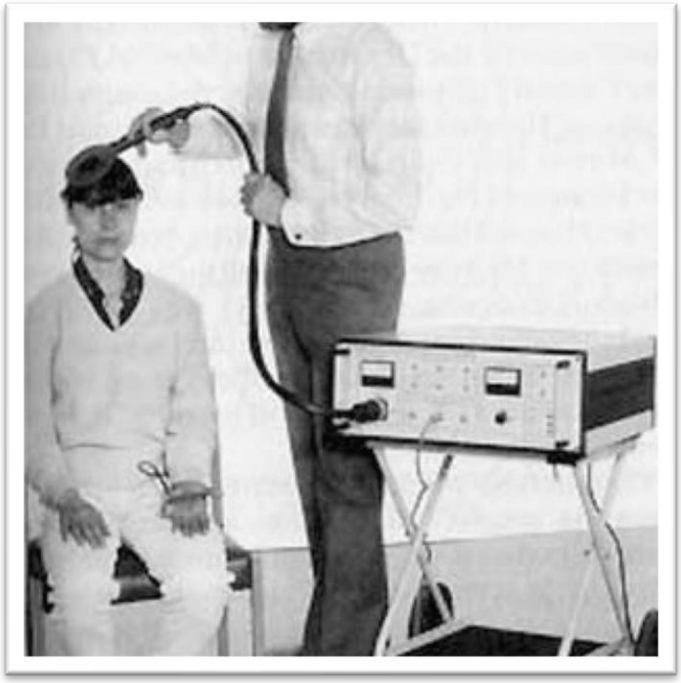

Figure 2. *Barker's first modern TMS equipment*

The investigators placed an excitation coil on the scalp of a healthy subject over the motor cortex and recorded twitch muscle-action potentials from the contralateral abductor digiti minimi muscle using conventional skin-surface electrodes. A clear and reproducible muscle contraction was observed and no discomfort was reported, so the first report describing stimulation of the brain was published soon thereafter in May 1985(9) .

Since then, the scientific interest for transcraneal magnetic stimulation increased exponentially, both in clinical and research setting. The first published clinical investigations using magnetic stimulation described results obtained from patients with multiple sclerosis and motor neuron disease and clearly demonstrated prolonged latencies between the motor cortex and target muscles in the multiple sclerosis patients (10).

1.2 How TMS works

TMS induces electrical currents in the brain via Faraday's principle of electromagnetic induction. The rate of change of the generated magnetic field determines the induction of a secondary current in a nearby conductor. In a conventional TMS equipment, an electric pulse which grows to peak strength and diminishes back to zero in a short period of time (<1 ms), is sent through the conductive wiring within the TMS coil. The rapid fluctuation of this current produces a magnetic field perpendicular to the plane of the coil that rises (up to 2.5 T) and falls rapidly in time. This magnetic field passes with little resistance through the subject's scalp and skull and induces a current in the brain that flows in a plane parallel to that of the coil but in the opposite direction of the original current. A pictorial definition of TMS is "electrodeless electric stimulation of the brain" via electromagnetic induction. Electromagnetic induction follows the inverse cube law, being the power of the magnetic field exponentially reduced as the distance from the original current increases. So, the induced current in the brain also decreases rapidly with distance from the coil. Because of this, the majority of TMS stimulation is restricted to superficial layers on the convexity of the brain (1.5–2 cm deep from the scalp). Thus, superficial areas of the brain closer to the plane of the coil will always be exposed to greater induced currents than deeper brain regions.

In magnetic stimulation, an electric field is induced both inside and outside the axon(11). To generate neural activation, the induced field must differ across the cell membrane: When the field is uniform with respect to the cell membrane, no current will be induced, so, only if the field crosses a bent axon or it is perpendicular to it an

electrical flow is generated. In brief, the probability of an induced field activating a neuron is proportional to the rate of change of the field(12),

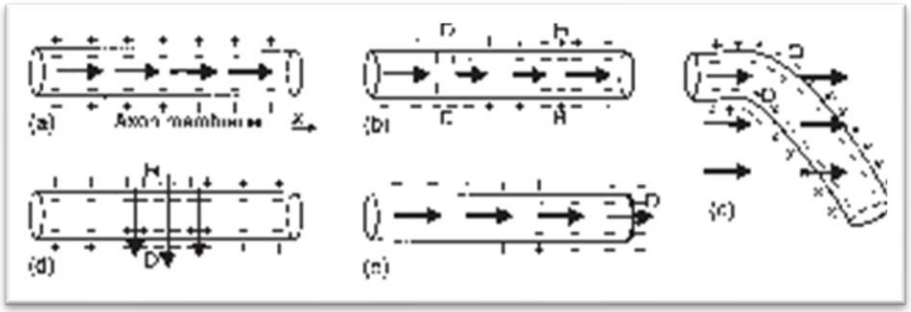

Figure 3. *Type of interaction between axons and the magnetic field*

Another extremely important issue is if TMS pulses activate or inhibit the neural tissue. This question arises from the facts that it can both induce movement or phosphenes (activation) and disrupt a movement or a cognitive process. Indeed, considering the mechanisms of TMS induction , it becomes readily apparent that TMS cannot be expected to distinguish between excitatory and inhibitory neurons within a region of stimulation, nor can it be expected to distinguish between orthodromic and antidromic direction of stimulation. So, classically TMS has been considered in two ways, depending on the use it was made of: disruptive, the mode of most interest to psychologists, it is believed that the noise produced is the final effector of the alteration induced in the task; activating, given the potentially facilitatory effect on the neuronal area under the site of stimulation (memory enhancement, motor skills, etc).

1.3 TMS hardware

A conventional TMS device has usually a common design and components that are almost universal. A TMS equipment consists of a main unit and a stimulating coil (Figure 4).

Figure 4. *Modern TMS hardware. MAGSTIM Rapid2 (MagStim Co, Wales)*

The main unit is usually composed of:

– Charging system—The charging system generates the current used to generate the magnetic field essential to TMS. A typical charging system can generate 8,000 A within several 100 ms.

– Energy storage capacitors—Capacitors allow for multiple energetic pulses to be generated, stored, and discharged in quick succession (typical voltage rating of 7.5 kV). Multiple storage capacitors are required for repetitive TMS protocols.

– Energy recovery circuitry—Energy recovery units allow for the main unit to recharge following discharge.

– Thyristor—Thyristors are electrical devices capable of switching large currents over a short period of time. In this case, the Thyristor acts as the bridge between the capacitor and coil, transferring 500 J between the two in less than 100 ms.

– Pulse-shape circuitry—Specialized circuitry can be used to generate either monophasic or biphasic pulses.

1.3.1 Type of coils

The stimulating coil consists of one or more well-insulated coils of copper wire (frequently housed in a molded plastic cover). As current passes through these coils, varied patterns of magnetic fields are generated which, in turn, generate a current in the opposing direction in any nearby conductor. Coils can be arrayed in a variety of shapes and sizes. The two types of coil in most common use are circular and figure-of-eight in shape (figure 5), and the regions of effective stimulation produced by these two configurations depend on the geometry of the coil and of the neurons underlying the coil and on local conduction variability.

Figure 5. *Most commonly used coils; A. round coil; B. Figure of 8 coil*

In figure 6 is shown the distribution of the electric field under a round coil

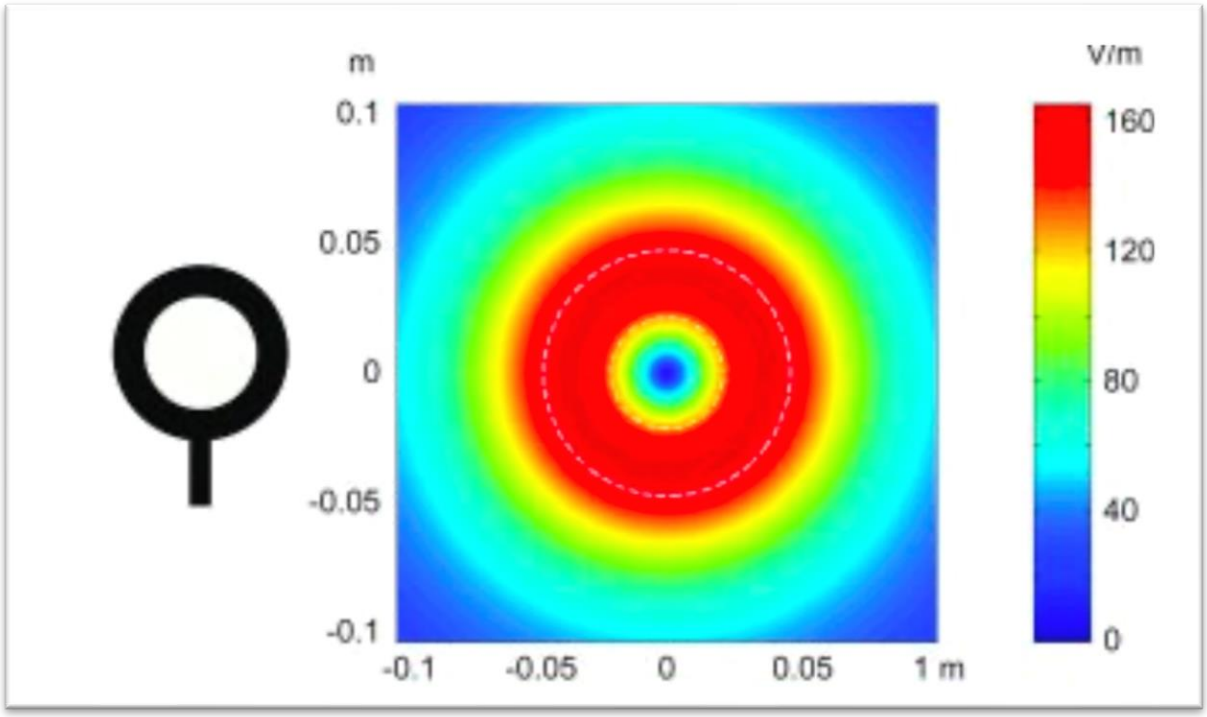

Figure 6. *Magnetic field generated by a typical circular coil*

Nerves lying tangential to any other part of the coil will be similarly stimulated. This does not mean that the effects of TMS are restricted only to the cortical area located precisely under the windings of the coil: First, the neurons receiving stimulation will activate neighbor neurons and also affect the organization of other connected neurons.

The use of a figure-of.8 coil increases the focality of stimulation (13). This configuration is of two circular coils that carry current in opposite directions, and, where the coils meet, there is a summation of the electric field (Figure 7) .

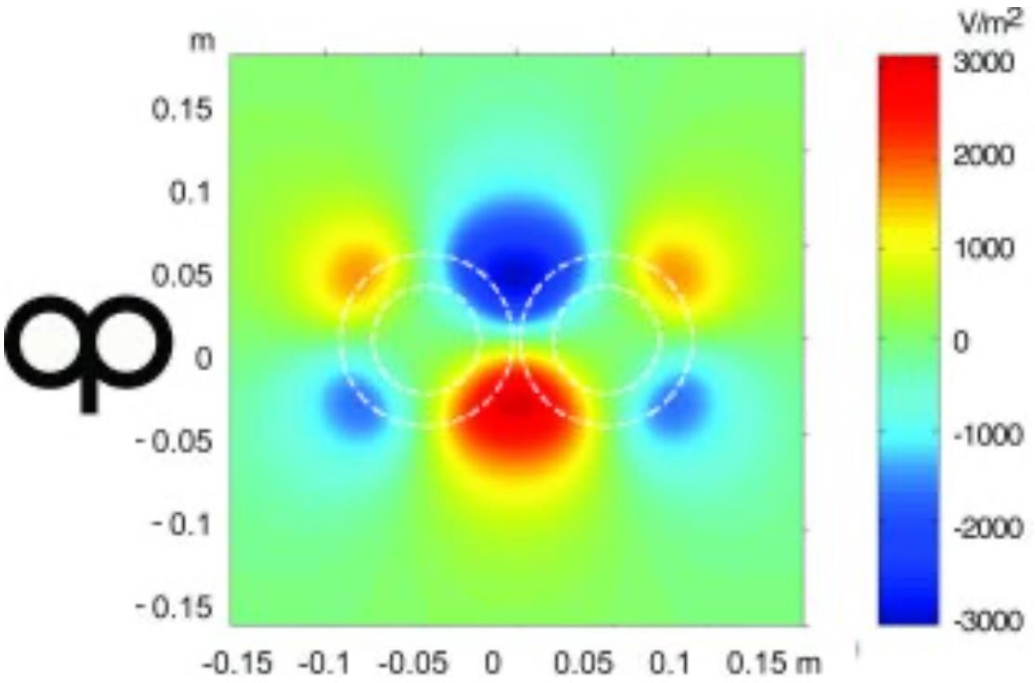

Figure 7. *Magnetic field generated by a typical figure of 8 shaped coil*

In addition to the new generated anode and cathode produced by the figure-of-eight coil, the two separate windings maintain their ability to induce a field under the outer parts of the windings. However, the outer parts of the coil are usually several centimeters away from the scalp and thus unlikely to induce effective fields, therefore increasing the probability that stimulation will be relatively focal.

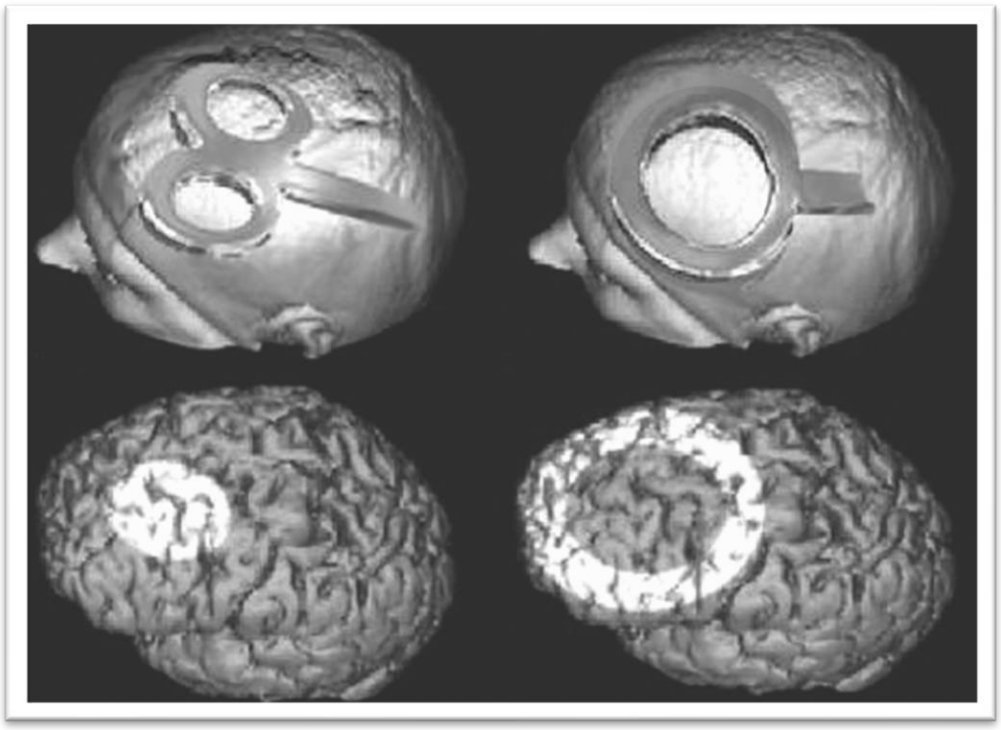

Figure 8. *Focality of the magnetic field in figure of 8 and circular coils*

The focality of TMS has been proved by many different classic works during the 90s. A part from the experiments of Barker and his group, that showed a selective stimulation of hand, face or leg, Marg, Meyer and Kastener (14) showed the appearance of phosphene more likely when the occipital cortex was stimulated, as well as Pascual Leone and collegues demonstrated the occurrence of a clear speech arrest if the face motor area or the frontal cortex of the dominant hemisphere was stimulated.

Even neglect and extinction can be elicited with focal stimulation of the parietal cortex(15) . Indeed, classical studies from Brazil-Neto and Wasserman(16) could map the motor cortex with EMG- recorded responses showing discrete representations of the fingers, hand, arm, face, trunk, and legs in a pattern that matches the gross organization of the motor homunculus.

However, in brief, the focality of TMS stimulation is influenced to a great extent by the shape and size of the stimulating coil and by the coil current waveform and amplitude. It is difficult to estimate the cortical territory influenced by TMS in a real brain, as this would require detailed information on the spatial distribution of the induced electric field within the head, the local anatomy, and the interaction between the induced field and the neural tissue .

Therefore, the area of activation cannot be simplified to a 2–3-cm^2 oval under the center of the coil. An idea of the focality achievable in practice can be gained by examining the motor responses evoked by TMS applied to the motor cortex. For example, just suprathreshold stimuli can preferentially activate small hand muscles with little activation of forearm musculature. Because of the physiology of axons, TMS is more likely to stimulate neurons that run parallel to the cortical surface. The depth of the penetration of the pulse is as important as the focality of the stimulation. Models of the electric field at different depths from the coil suggest that relatively wide areas are stimulated close to the coil, decreasing in surface area as the field is measured at distances farther from the coil. For a figure-eight coil, it is estimated that stimulation 5 mm below the coil will cover an area of approximately 7 by 6 cm. Given that the depolarization of the neurons is secondary to the electric field generated in the brain

more than the coil-generated magnetic field, it is indeed important to understand how this field is distributed in the brain.

1.4 The motor evoked potential

Transcranial magnetic stimulation of the brain induces muscle responses termed motor-evoked potentials (MEPs)(9). MEPs are widely used to study the physiology of corticospinal conduction in healthy subjects and in patients with diseases of the central nervous system(17). A variety of parameters of MEPs can be studied(18), including the latency , the central motor conduction time (CMCT), the size of the MEP (amplitude, duration and area), and others (such as stimulation thresholds, silent period, facilitation).

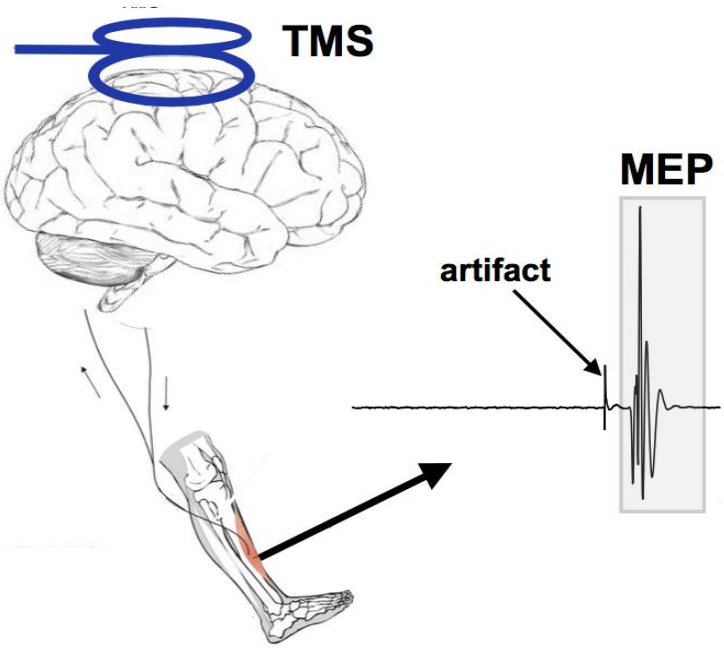

Figure 9. *Normal MEP generated by a TMS pulse on the motor cortex*

If electrical stimuli of increasing intensity are applied to a peripheral nerve, the amplitude of the compound motor action potential (CMAP) increases, until the size will not increase if the stimulus intensity is further increased. This reflects the fact that all the axons of the nerve have been stimulated and no further muscle fiber can be recruited. The same happens for the MEP although, even if a strong impulse is applied, the size of the MEP will usually not reach the same size as the CMAP to maximal peripheral stimulation(19). This is due to various factors that can influence the size of the MEP:

- the number of recruited motor neurons in the spinal cord
- the number of motor neurons discharging more than once to the stimulus
- the synchronization of the TMS-induced motor neuron discharges.

In turn, this is secondary to further factors influencing motor neuron activity. The amplitude of a MEP not only depends on the localization of the coil, but also on the direction of the induced electrical field. For instance, clockwise current orientation within a circular stimulating coil centered over the vertex led to preferential stimulation of the right hemisphere, while counterclockwise current flow preferentially stimulated the left hemisphere. Moreover, the MEP is influenced by the excitability of the corticospinal pathway, which is variable and can be facilitated by a number of mechanisms. Facilitation by voluntary background contraction is observed in all target muscles, and with both electrical and magnetic transcranial stimulation. The increase in the amplitude of the MEP that occurs in response to voluntary contraction is probably mainly caused by an increasing number of spinal motor neurons brought to fire by the

transcranial stimulus, since desynchronization of the motor neuron discharges appears to be unchanged by voluntary contraction. TMS over the motor cortex preferentially activates pyramidal neurons via trans-synaptic inputs from excitatory interneurons, (Figure 10) likely through activation of cortico-cortical axons (20,21). With a monophasic magnetic stimulator, the lowest threshold occurs when the direction of the induced electrical current within the cortex flows from posterior to the anterior direction, perpendicular to the line of the central sulcus. Pivotal studies demonstrated that TMS pulses applied in such a way near threshold produces a single I-wave (I1) without a preceding Direct (D) wave (22–24).

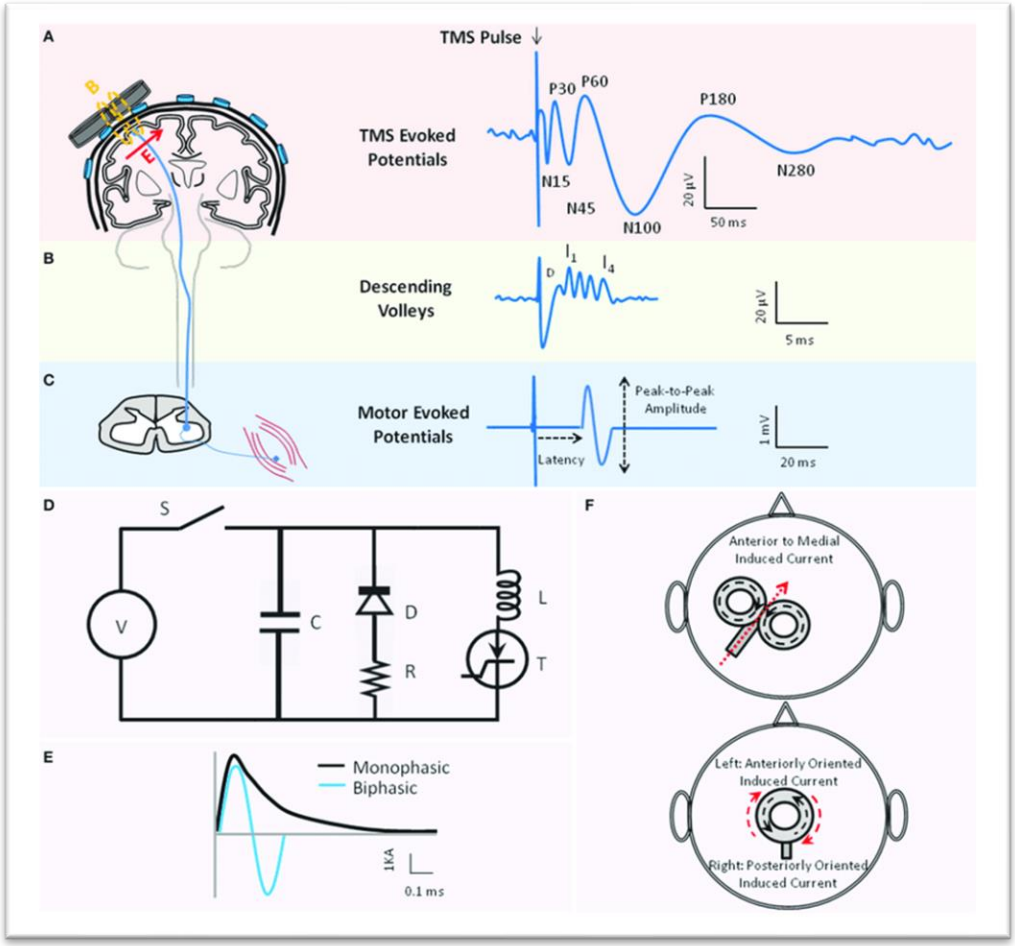

Figure 10. *Resume of cortical mechanism and induced current of a typical TMS equipment*

Even a slight positional change or rotation of the coil on the scalp can alter the size and latency of the MEP significantly, in particular when using a focal figure-8-shaped coil. If the coil is rotated so that the induced current direction is from lateral to medial, along the line of the central sulcus, both D- and I-waves may be elicited near threshold. When high stimulation intensities are used TMS pulses will result in formation of both D and I waves. At the level of the spinal alpha motor neuron, the descending D- and/or I-wave volleys result in progressive depolarization until there are action potentials. Whether the initial part of the descending bursts (D- or I1-wave) or later waves (I2 or higher) will activate the motor neuron depends not only on the strength and number of the descending volleys, but also on the excitability status of the spinal alpha motor neuron. During voluntary muscle contraction of the target muscle, the resting potential of inactive spinal motor neurons is closer to threshold, and a single descending volley may provide sufficient synaptic input to discharge the spinal motor neurons. When relaxed, several descending volleys may be needed to provide the necessary temporal summation for motor neuron firing. Voluntary muscle contraction therefore provides facilitation for MEP recordings: MEP latency is reduced by 2-3 ms compared to the relaxed state; MEP amplitude is larger; and MEP threshold the intensity of cortical stimulation needed to evoke a motor response is reduced (25–27).

1.4.1 How to record a motor evoked potential

For routine MEP studies, the magnetic stimulator is connected to a standard electromyography (EMG) machine to synchronize the recording with the TMS pulse. For upper limbs MEP the swap line has to be set at 50-80 ms while 100-120ms for the legs. A higher value is needed if the cSP (cortical silent period -see further)PM has to be

analyzed (300-500ms). To ensure the pre-stimulus muscle relaxation (or to quantify the EMG activity pre-cSP recording), a delay of 20-50 ms can be used. MEPs are usually recorded with surface electrodes in a belly-tendon configuration taped to the skin overlying the target muscle, with the same configuration used for CMAP recording. Filter setting should be relatively open (~1-2000 Hz), and a low-pass filter of 1 Hz is recommended to minimize the duration of the stimulus artifact during magnetic stimulation (17). The subject should be seated comfortably. During MEP recordings, the subject should be relaxed with eyes open. It has been recommended to ask the subject to perform simple calculations (such as subtracting serially 7 from 100) in order to minimize threshold and MEP amplitude variability (28,29). To avoid excessive coil movement, and to ensure the reproducibility of the recording, after localizing the optimal stimulation site, the coil position is usually marked with a pen on the scalp and used for the remainder of the testing for this muscle. For routine diagnostic TMS studies, using a large circular coil (diameter 8-12 cm) a large volume of brain tissue can be activated, resulting in non-focal stimulation. It is suitable for MEP recordings from upper and lower extremities. For more focal stimulation, a figure of 8-shaped coil is usually used. A small figure-8-shaped coil is used primarily for focal stimulation of the motor cortex hand area (such as for mapping studies). Stimulating the lower extremity motor cortex is facilitated by using a figure- 8-shaped coil with large wings that are slightly angulated, providing a very strong magnetic pulse penetrating deeply into the cortex. The recommended stimulation site for MEP recording of the upper extremities is Cz (the intersection of the nasion-inion and tragus-tragus lines) flat on top of the head This position can be used for both distal and proximal upper-extremity recordings. However some authors suggest the placement of the coil center at a point 2 cm

posterior and 2 cm lateral to Cz (overlying the target hemisphere) for optimal stimulation of the first dorsal interosseus muscle (large round coil with 11.6 cm outer diameter) (30). The optimal stimulation site for each individual may be determined by a slight variation of coil positioning until the site with lowest MEP threshold is identified (30). A practical approach is to stimulate at vertex and then 1 cm away in the four quadrants. MEP threshold when stimulating at the hotspot is likely lower than when using the standard circular coil position, but MEP amplitudes at higher stimulation intensities and MEP latency will not differ significantly(31), unless a figure of 8 coil is used, when the hotspot searching is mandatory to ensure that the MEP with the shortest latency and higher amplitude is identified.

The coil current direction is important for monophasic stimulators. For upper-extremity TMS of the right hemisphere, the current direction within the circular coil needs to be clockwise so that the induced cortical current (with opposite direction) is perpendicular to the precentral gyrus in posterior-anterior direction, and vice versa for the left hemisphere. With biphasic stimulation, the current direction is not important and a large circular coil at vertex activates both hemispheres simultaneously. When a figure-8-shaped coil is used for upper-extremity recordings, the center of the coil should be directly over the target region in the motor cortex. The handle is often pointed backwards (32,33). Largest responses are obtained when the coil axis is ~45-50° to the parasagittal plane with a backward-flowing current in the coil so that the induced current in the brain is perpendicular to the precentral gyrus flowing posterior-anteriorly (16,17,19,34). For the lower extremities MEP recording, because of the mid line location of the corresponding motor cortex, the optimal stimulation should be latero-lateral perpendicular to the midsagittal line. This is achieved by placing a large circular

coil ~2-4 cm anteriorly to Cz, so that the posterior segment lies midline over the precentral leg region. A large figure-8-shaped coil with the center near Cz or slightly posteriorly can be also used for leg recordings due to its more focal and deeper penetration than a circular coil, large angulated double cone coils often have the handle pointing upwards toward the ceiling allowing a deeper stimulation of the parasagittal areas (35). Finally, for the facial muscles, according to the somatotopic organization of the motor cortex, the stimulation area is located few centimeter lateral to the hand region with the same coil orientation used for the hand. The only exception is for the masseter muscle that needs a parallel rather than perpendicular current to the precentral gyrus(36,37).

1.4.2 MEP threshold

MEP or motor threshold is the lowest stimulus intensity of TMS that gives a recordable MEP in a target muscle. The motor threshold is usually determined at the beginning of the MEP recordings, as it provides a reference for setting the stimulation intensity for recording other parameters. A common definition of the MEP threshold at rest (resting motor threshold, rMT) is the stimulus intensity required to elicit reproducible MEPs of ~100 μV in 50% of 10 consecutive trials(19). Usually the measurement is started below the expected threshold intensity increasing the stimulator output progressively by 5% steps (absolute percentage of stimulator output) (19), and then near threshold successively decrease the stimulation intensity in 1% or 2% steps until <50% of 10 stimulations produce a measurable response . Many different ways of measuring the rMT have been proposed during the last years (PEST, threshold hunting, cSP) although the most used in the majority of the laboratories is still the Rossini-Rothwell method.

Using large round coils for stimulation and recording from abductor digiti minimi (ADM), MEP threshold was 47.7 ± 7.5% with a 1.5 T coil, and 41.2 ± 7.3% with a 2 T coil(38) . In longitudinal studies, the test-retest MEP threshold difference was 2.5 ± 2.6% of the stimulator output, and a threshold change of 10% was considered pathological (38). Side-to-side variation should be <5% (34).

MEP threshold is generally lower for distal than proximal muscles; lowest threshold values are reported for intrinsic hand muscles and finger extensors, in keeping with their large cortical representation (28,39) . Lower-extremity muscles and pelvic muscles have higher thresholds. MEP threshold varies widely in the healthy population, with a slight increase with aging. There is no significant difference by gender (39,40). A lower threshold has been reported for the dominant hemisphere by some authors and handedness should be documented(38,41,42), but any difference between the two hemispheres, if at all present, is physiologically minimal.

1.4.3 MEP latency

The latency of the MEP is the time intercurrent between the cortical stimulation and the onset of an evoked potential in the target muscle. It depends or whether the muscle is at rest or during activation. Often, MEP recordings for latency and amplitude measurements are done with facilitation for practical reasons: it is usually difficult to achieve complete muscular relaxation in patients; more importantly, during facilitation, MEP threshold is reduced and amplitudes are greater, so less stimulation intensity is needed, especially when the lower limbs are studied. MEP latency during facilitation is typically 2-3 ms shorter than during complete relaxation, sometimes up to 6 ms (28), and recording during muscle contraction results in the shortest reproducible MEP

latency. In practice, the subject is asked to moderately contract the target muscle for a few seconds and the TMS pulse is applied when the subject maintains a tonic contraction. In between each stimulus, the subject rests in order to avoid fatigue. Most investigators use values between 10% background muscle contraction and 20% of maximal voluntary force. Appropriate muscle contraction may be achieved by pinching thumb and fingers against each other for intrinsic hand muscles, or by making an isotonic muscle movement against gravity and without resistance (proximal arm muscles and leg muscles). Higher degrees of muscle contraction should be avoided, since excessive EMG background makes the identifying MEP onset rather difficult (28). There are cases when the subject cannot maintain a steady low level muscle contraction in the target muscle (hemiplegia from cerebral infarction p.e.), then other techniques may be used, including reflex activation, contraction of contralateral muscles or facial muscles. Even the mental imagery of a movement is known to produce facilitation in MEP generation. Other facilitatory maneuvers include vibration of the examined muscle (28) or pre-stimulation of the mixed nerve innervating the target muscle (43) . The stimulation intensity used when the latency is the main aim of the study should be well above the MEP threshold. When using low stimulation intensities close to threshold, MEP latencies of repeat trials are more variable, and higher stimulation intensities should therefore be used for clinical testing (44). It is described that the stimulation intensity should be of 120-150% of the resting motor threshold (19) , although various other techniques and normal values are published according to different methods of MEP generation (23,45,46).

1.4.4 Central Motor Conduction Time

MEP latency is the time from the motor cortex stimulation to MEP response in the target muscle and therefore includes both a central component (time from cortex to activation of the lower motor neurons) and a peripheral component (time from activation of the spinal motor neurons to the muscle response). In clinical and experimental practice the central motor conduction time (CMCT) is used as a more sensitive measure of pyramidal tract function by subtracting from MEP latency the time for the peripheral segment, the peripheral motor conduction time (PMCT), following the formula CMCT = MEP latency - PMCT.

The PMCT can be measured by different modalities (figure 11):

1. F wave: is a conventional electrical stimulation of the peripheral nerve innervating the target muscle and measuring the minimal F-wave latency of (usually) 20 recordings. The peripheral motor conduction time PMCT-F is then calculated as PMCT= (Fw+ M -1)/2 where F is the minimal F-wave latency, M the latency of the compound muscle action potential or M-wave, and 1 (in ms) is the estimated turnaround time for antidromic activation of the spinal motor neuron (Rossini et al. 1994). This method is only applicable for muscles and nerves with recordable F-waves (distal muscles in hand and foot).
2. Spinal nerve root magnetic stimulation: the coil is placed either on the seventh cervical vertebral apophisis or the lumbar point (L1/2) and a TMS pulse is delivered. According to the direction of the stimulus administered the right or the left roots are preferentially stimulated. Using this technique, the nerve roots are typically stimulated in the region of the intervertebral foramen. The PMCT

> therefore does not include conduction time from anterior horn cells to the intervertebral foramen. As a result, the calculated central motor conduction time is estimated as slightly too long, as it includes this proximal segment of the spinal nerve root.

According to this, whenever there is focal slowing across the proximal root motor segment, the CMCT measurement with the spinal root stimulation might result in a false positive, given that the same portion of motor root is included in the central and not peripheral conduction time (i.e. peripheral nerve disorders and lumbar radiculopathies due to disk herniation and lumbar spinal stenosis). This does not occur with F-wave method. On the contrary the advantages of magnetic root stimulation vs the F-wave method include the ability to record from proximal and distal muscles, often simultaneously. In addition, F-wave latencies are inherently more variable and thus a larger number of electrical stimulations are needed for this method to obtain a reliable minimal F-wave latency. It may also become difficult in peripheral nerve disorders to obtain F-waves at all.

There is a slight increase of CMCT with age, but the correlation is weak and may be neglected for routine studies (34). Kloten et al. (1992) (30)found that the latency difference between age groups 19-29 and 60 years was 0.1 ms for the biceps brachii, 0.7 ms for the first dorsal interosseus (5.8 ± 1.0 ms vs 6.5 ± 1.1 ms), and 2.1 ms for the tibialis anterior muscle (14.0 ± 1.3 ms vs 16.1 ± 1.9 ms).

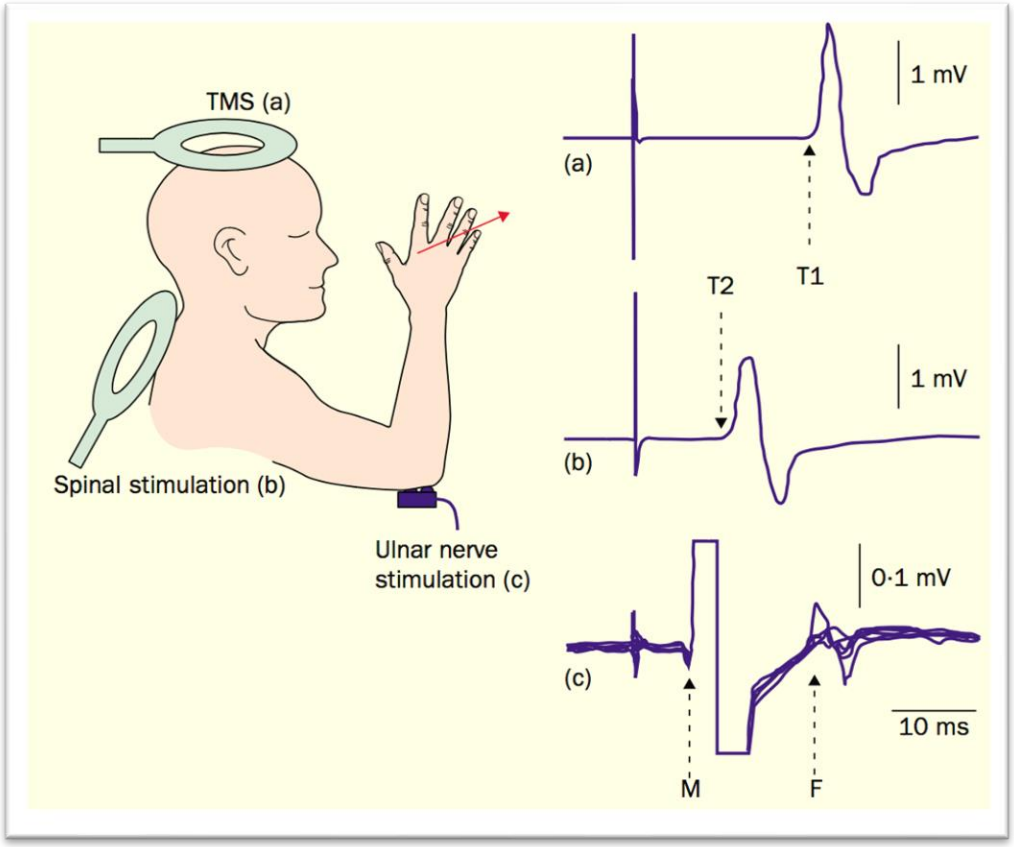

Figure 11. *Central motor conduction time typical study procedure*

1.4.5 MEP amplitude

The absolute MEP amplitude reflects both upper and lower motor neuron activity and is affected by peripheral nerve disorders. Correlating MEP amplitude with the amplitude of the compound muscle action potential (CMAP) that is obtained by conventional electrical nerve stimulation of the peripheral nerve is a more useful measure of upper motor neuron function. It estimates the portion of the pool of spinal motor neurons that is activated by TMS (28) .

1.5 The cortical silent period

Calancie et al (47)were the first to show that transcranial magnetic stimulation (TMS) of the motor cortex in humans produces not only a muscle twitch in voluntarily activated contralateral limb muscles but also a subsequent period of electromyographic (EMG) suppression, lasting up to 100-300 ms. Investigation of the cSP can be easily performed: a single suprathreshold TMS pulse is applied to the motor cortical representation of a tonically preactivated target muscle, thereby producing a period of EMG silence in contralateral small hand muscles. The physiological basis of cSP have been deeply investigated and the mechanisms include various steps; at the spinal level a suprathreshold TMS pulse delivered to the motor cortex provokes the activation of spinal motor neurons and of Renshaw interneurons which are inhibitory neurons, furthermore fibers descending from the motor cortex can activate spinal inhibitory Ia-interneurons; at supraspinal level the TMS pulse activates both facilitatory and inhibitory neurons within the motor cortex.

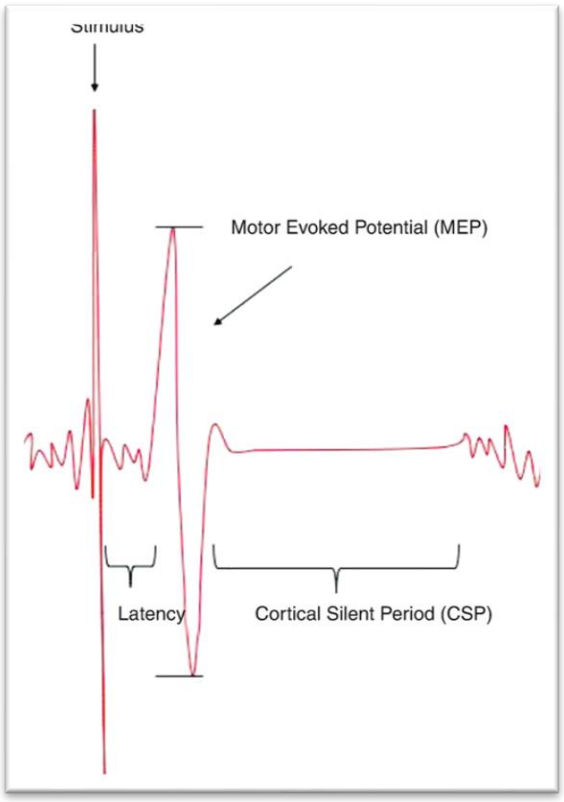

Figure 12. *MEP and CSP after a TMS stimulus*

It has been demonstrated that the cSP induced by TMS can be elicited at low stimulation intensities in the absence of a preceding MEP (48,49). Inhibition in the absence of corticospinal excitation underlines the cortical origin of the inhibition. Evidence for a cortical origin of the cSP arises from the finding of an abolished cSP in stroke patients suffering from isolated ischemic lesions within the primary motor cortex(49,50). Despite the lack of cSP, the MEP and the spinal silent period were preserved, indicating a strong contribution of inhibitory motor cortical mechanisms even to the early part of cSP. In summary, it is now generally agreed that spinal inhibitory mechanisms contribute to the first part of the cSP up to 50 ms, but its later part is generated exclusively by inhibition that originates within the motor cortex. Therefore, the cSP can be considered as a probe of motor cortical inhibition. cSP can

give a measure of the speed with which the motor circuits can resume their normal interaction after an artificially-induced focal disruption

1.6 Safety and side effects of TMS

Transcraneal magnetic stimulation is safe when adequate precautions are taken(51). Contraindications to TMS are mostly related to the exposure to the magnetic field, similar to those for magnetic resonance imaging (and apply to both the patient and the examiner). The large magnetic pulse may damage electronic devices, and metal objects will be subject to mechanical forces and may become hot. The subject needs to be asked specifically about the following exclusion criteria before proceeding with magnetic stimulation:

1. Implanted metal devices such as cardiac pacemakers or defibrillators, intrathecal drug delivery pumps, or spinal cord, vagus nerve, or similar stimulators

2. Acoustic devices such as cochlear implants.

3. Presence of intracranial metal such as aneurysm clips that might be dislodged by high-intensity TMS. The physician should ask for prior neurosurgical procedure.

4. History of epileptic seizures. There are a few reports of seizures occurring at or shortly after single-pulse magnetic stimulation, mostly in patients with epilepsy (52–54) but also in a patient with multiple sclerosis (MS)(55) and bipolar disease(56) . One patient with a large middle cerebral artery infarction developed the first seizure during TMS and subsequently required anticonvulsant treatment for epilepsy (52). There is no report of single-pulse TMS inducing a seizure in a normal subject. The history of seizures is not an absolute contraindication for single-pulse TMS, and single and repetitive TMS

techniques have been used successfully in the study and treatment of epilepsy (53,57) and without worsening of epilepsy.

Other safety concerns relate to the high-intensity impulse noise artifact associated with the discharge of the magnetic coil that is often placed in close proximity to the ear(51). As of today, no acoustic damage has been reported in humans after single pulse exposure. However, earplugs are usually used to prevent potential damage. The use of single pulses greatly reduces the impact of the brisk noise on the human ear, compared to repetitive stimulation.

Moreover, local transient discomfort on the skin under the stimulation area is reported. It is due to involuntary contraction of the scalp muscles.

Another frequent side effect is mild headache during or after prolonged stimulation studies that is usually self-limited and can be treated with analgesics. There is no evidence of any significant adverse effect of single TMS on cognition, hormone release, or the cardiovascular system. Before beginning the examination, both the patient and the operator(s) should also move watches and magnetic-sensitive devices (credit cards etc.) to a safe place at least 50 cm away from the magnetic coil (28).

1.7 The human motor system in brief

The human motor system consists of cortical, subcortical, and spinal structures that with an extremely fine tuning are able to produce a volitional (or unconscious) movement(58). The final effector is the primary motor cortex (M1). It is located bilaterally in the brain on the posterior part of the frontal lobe (i.e., anterior wall of the central sulcus), termed Brodmann area 4. The M1 microstructure is arranged into multiple cortical layers (i.e., I-VI) characterized by their cellular composition. The defining feature of M1 from other neocortical regions is it contains giant pyramidal neurons of Betz in layer V.

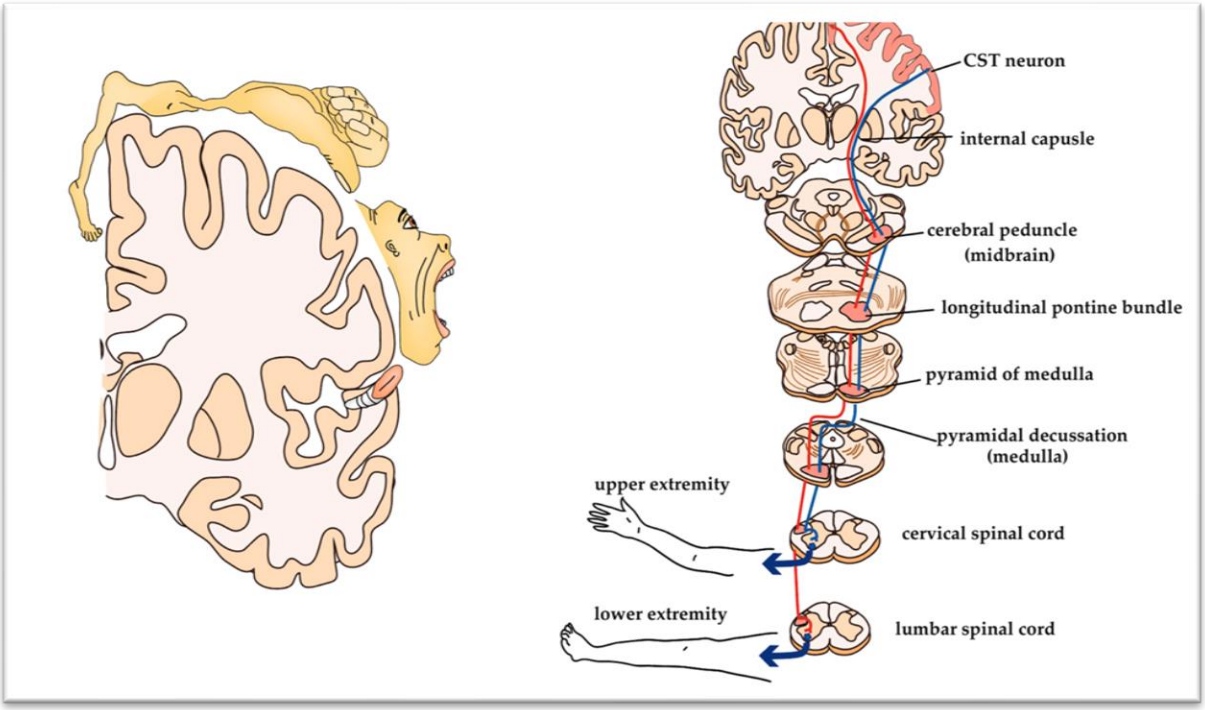

Figure 13. *Motor system in brief*

Different other cortical structures form the human motor system, including the premotor cortex (PM) located immediately rostral to each M1 and the supplementary motor area (SMA) on the medial aspect of each hemisphere rostral to M1 (i.e.,

Brodmann area 6). Both areas are involved in the motor plan production that is subsequently forwarded to M1 to generate a volitional movement. Once generated, the command is transmitted by the descending motor pathways down to the to α-motoneurons (αMN) and interneurons in the spinal cord. In humans, the lateral corticospinal tract (CST) is considered the primary descending motor pathway to execute voluntary movement. The lateral CST originates from M1, PM, SMA, cingulate motor areas in the frontal lobe, and somatosensory cortex in the parietal lobe, with the highest proportion of fibers originating from M1(59–61) . The CST is mainly formed by axons of the large and small pyramidal neurons, it descends from the cortex and enters the internal capsule where it travels caudally passing through the midbrain in the cerebral peduncle, the basilar pons, and forms the medulla pyramids. At this point, approximately 75 – 90% of corticospinal axons decussate (62–64) and continue to descend caudally via the dorsolateral columns of the spinal cord to innervate αMNs. The remaining uncrossed axons descend via the ventral column forming the ventral CST, also referred to as the ipsilateral 'uncrossed' CST.

Descending axons can act directly on αMNs or indirectly via spinal interneurons. The crossed portion of the CST innervates motor nuclei in the dorsolateral column and interneurons in the intermediate zone of the spinal cord. The main function of the lateral 'crossed' CST is to control distal musculature, especially dexterous movements with the hand. The ipsilateral 'uncrossed' portion of the CST projects bilaterally to the ventromedial column and portions of the intermediate zone. The main function of the ventral CST is control of the neck, trunk, and proximal upper limb (65). Another lateral pathways of the motor system include the corticobulbar tract that descends with the CST through the internal capsule, midbrain, and brain stem, to innervate cranial nerve

motor nuclei in the brainstem. Indeed, the presence of a communication pathway between the two hemispheres of the brain is essential for coordinating goal-directed movement with the upper limb, especially during bimanual movements. The corpus callosum is the principal structure connecting homologous cortical areas to permit interhemispheric communication. The left and right cortical motor areas are connected via callosal motor fibers in the posterior midbody and isthmus of the corpus callosum. The corpus callosum is somatotopically organized, with the hand area more anterior and ventral to the foot area (66,67). The corpus callosum is typically assumed to be an inhibitory projection, whereby unilateral upper limb movement inhibits the contralateral M1 to prevent mirroring with the opposite upper limb(68) . However evidence also shows the corpus callosum may have excitatory connections; therefore it is possible that it is both an excitatory and inhibitory pathway (68,69).

1.8 Transcranial magnetic stimulation and stroke

In the acute stage of stroke it is common to find reduced amplitude or absent MEP and prolonged CMCT over the affected hemisphere. In patients tested within 24 h of stroke onset, ~20% have absent MEPs, usually in subjects with severe motor paralysis (70,71) In less severely affected patients, MEPs often have reduced amplitude, prolonged latency, and increased threshold (72–74). Many studies have indicated prognostic value of early TMS studies in predicting outcome in ischemic stroke patients (70,75–79). In general, the presence of MEPs in the acute phase correlates with a good functional recovery in most reports, whereas absent MEPs predict poor function. In a large study of 118 patients with first-ever stroke of mixed etiology and location who underwent TMS within 72 h and serially up to a year, patients with MEPs present on initial testing had consistently higher functional scores throughout the study and better recovery at 12 months, whereas those with absent MEPs had high probability of death and poor functional outcome (74). Interestingly, the patients with prolonged CMCT recovered more slowly than those with normal CMCT, but were similar after 1 year. In another study, patients with normal CMCT recovered better by 6 months than those with delayed CMCT, and the presence of MEPs provided information on motor recovery regardless of initial strength(76).

The recovery of MEP latency on serial testing is highly correlated with return of muscle strength and hand function scores (80). MEP testing was more sensitive than clinical examination to detect residual corticospinal function, and nearly all muscles with present MEPs on initial testing eventually presented a consistent motor recovery(79). They reported correlation between abductor digiti minimi MEP amplitude and the hand

motor score 6 months later, and between biceps brachii amplitude and the subsequent arm motor score, but no clear correlation between MEP from leg muscles and lower-extremity motor scores. MEPs are best recorded during facilitation (by voluntary contraction of target muscle, or if not possible contralateral muscle), as MEPs are often absent at rest in affected limbs of acute stroke patients who may have good outcome (74). On the other side, some degree of motor recovery may occur in some patients with absent MEPs on initial evaluation, even when assessed during facilitation(79). About topographic localization, MEPs recorded from patients with superficial (cortical) infarcts have smaller amplitudes and longer CMCTs than those in deep infarcts, in correlation with worse prognosis in the cortical infarct group (71). In this classical study, consecutive MEP recordings over 3 months showed gradual recovery of MEP amplitudes, greater for the deeper infarcts, whereas CMCT changes over time were generally variable. Again, those classical studies showed that MEPs were evenly absent in upper- and lower-extremity muscles at basal measurements, with subsequently earlier recovery in proximal arm muscle (biceps) and in lower-limb muscles than in hand muscle (71). Normalization of MEP is greatest in the first 80 days, but can progress over many months (74,81,82) .

The cSP duration is often prolonged in patients with cerebral infarcts(83–85) , but may also be shorter than normal in patients with cortical infarcts (86) . The length of the cSP duration on day 7 had predictive value in one study: patients with good recovery on follow-up 3 months later and normal controls typically had longer SPs with increasing stimulation intensity, whereas in acute patients with subsequently poor outcome and in chronic patients with spasticity there was shortening of the cSP with increasing volitional isometric muscle contraction (87). Ipsilateral MEPs are sometimes present in

stroke patients when stimulating the unaffected cerebral hemisphere and recording from the paretic muscle. These ipsilateral MEPs are more easily elicited in proximal than in distal muscles, and may have poor prognostic implications according to some authors(88), or be important for recovery(89) .

1.9 Stroke and motor cortical plasticity

After suffering a stroke the brain goes under a series of adaptative mechanisms that induces changes in both cortical and subcortical matter which usually result in a recovery of the lost function(90).

1.9.1 Cellular plasticity in the perilesional area

A number of cellular changes have been observed in animal models of stroke(91). At the core of the lesion there is an area of irreversible neuronal death, but immediately surrounding this, there is an area called the penumbra where if blood flow is properly restored within a certain amount of time, these neurons could be partially or fully restored. The non-ischemic area close to the lesion is called peri-infarct area, and this is extremely important for neuronal plasticity. In those areas, several changes are observed such as neuronal hyperexcitability, axonal sprouting, dendritic modeling and new synapses generation(92,93).

1.9.2 Reorganization of neural networks in the ipsilesional and contralesional hemispheres

Cellular changes occur not only in the area immediately surrounding the lesion, but also in other brain areas(94,95). Seven days after a stroke, brain slices from rats show reduced intracortical inhibition of cells in the contralesional hemisphere and unaffected areas of the ipsilesional cortex (96). The resulting hyperexcitability may assist with

reorganization in these areas after stroke. Human studies which probe the brain at a network level support this idea. For example, early after stroke TMS studies show a decrease in corticomotor excitability from the ipsilesional M1 (i.e. MEP amplitude is reduced and motor threshold is increased)(97). However, within the ipsilesional hemisphere, paired-pulsed TMS studies show a decrease in intracortical inhibition which promotes excitatory activity within this hemisphere and supports the changes observed in brain slices from rats(98). Over the first four months after stroke, corticomotor excitability in the ipsilesional M1 increases (i.e. MEP amplitude increases and motor threshold decreases). This increase in ipsilesional M1 excitability correlates with improvements in function, which might suggest the consequent facilitation of the networks during motor recovery.

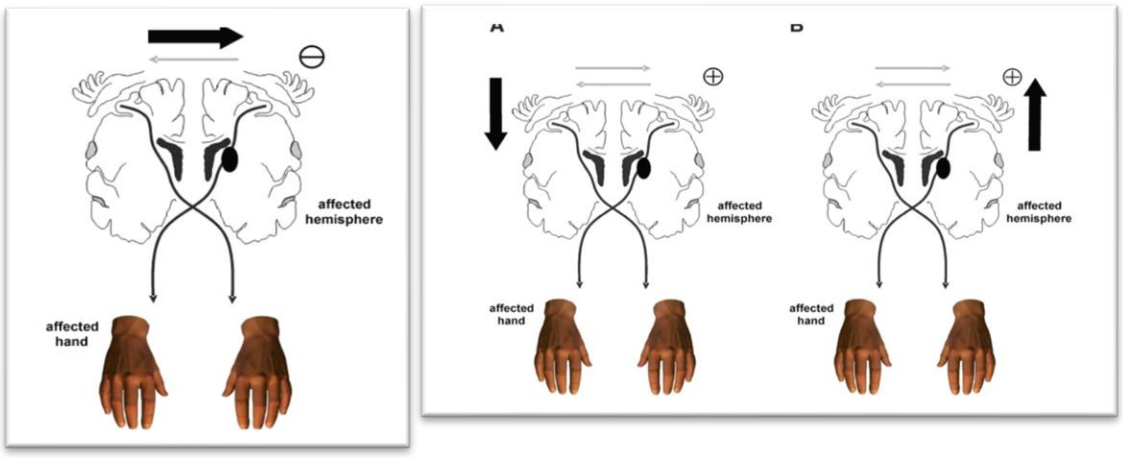

Figure 14. *Interhemispheric imbalance in stroke patients*

Imaging studies, such as functional magnetic resonance imaging and positron emission tomography have observed the level of neural activation in various brain areas when a subject with stroke is moving the hemiparetic limb, compared with healthy controls(99) . Firstly, overactivation, defined by higher relative cerebral blood flow compared with

healthy controls, has been observed in the peri-infarct cortex of an M1 infarct, which could be translated to increased activation in this area. Secondly, there is overactivation of ipsilesional motor and non-motor areas . This increased activation in the ipsilesional S1/M1 has been associated with better recovery after stroke. Thirdly, there is overactivation of the contralesional S1/M1, and of secondary motor areas and non-motor areas in the contralesional hemisphere. Overactivity of the contralesional M1 has been associated with the use of the ipsilateral cortico-spinal pathway not decussating.

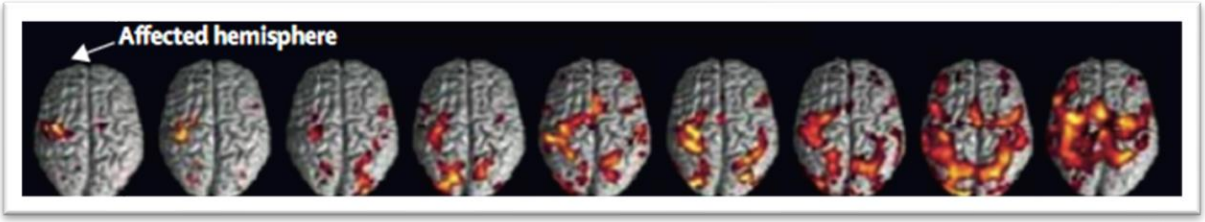

Figure 15. *Vicariation of multiples brain cortical areas after stroke (timeline from left to right)*

Animal studies have explored the reorganization that takes place within the ipsilesional S1/M1 after stroke. This involves changes to the somatotopic organization of the S1/M1 shown to decrease by four months post-stroke, as activation of the ipsilesional S1/M1 which is associated with axonal sprouting(100).

Human studies, using TMS to discern which areas of the M1 innervate different body parts, have shown the dynamic process occurring after a stroke. For example, the representation of the hand on the ipsilesional M1 is initially smaller after stroke compared with the contralesional side, but an increase in this representation is associated with improved recovery(101) (Figure 15).

All the studies reported support the idea that functional recovery after stroke relies on the reorganization of undamaged areas in both hemispheres, and particularly the ipsilesional S1/M1, so that these areas can take over the function of the lesioned area; this is also known as the vicariation model for recovery(102).

1.9.3 Interhemispheric inhibition

On the other side, the interhemispheric imbalance model, suggests that a stroke disrupts the balance of inhibition between the two hemispheres, and that lowered excitability in the ipsilesional hemisphere is in part related to excessive interhemispheric inhibition from the contralesional to ipsilesional hemisphere. This model has led to interventions which aim to increase ipsilesional excitability by inhibiting the contralesional hemisphere and in turn reducing interhemispheric inhibition(103)

1.10 Sensory threshold

Sensation is commonly impaired immediately after stroke. In the acute and early sub-acute period conscious touch perception is reduced by up to 85% on the more-affected side, and 25% on the less-affected side(104). Changes in cutaneous sensation may occur isolated or accompanied by altered proprioception or global sensory and motor dysfunction. Impairments are defined as ‘‘problems in body function or structure that result in a significant deviation or loss’’. Body functions and structure in the periphery, such as somatosensory receptors and their afferent pathways are not directly damaged by stroke, rather the nature of sensory loss is related to lesion location(105). However, secondary deficits may occur subsequently in healthy neurons remote but functionally coupled to the focal lesion through diaschisis and cortical reorganization. The

prevalence of long-term sensory deficits at 6 months post-stroke has been estimated at 37%, although the impairments were not quantified or described (104,106).

Reduced sensation is associated with poor recovery of physiological function related to movement, referred to here as motor-function. These changes in motor-function may include slower improvements in the hemiplegic arm, delayed movement onset and impaired reach trajectories. Consistent relationships between reduced sensation in different modalities (such as cutaneous or proprioceptive sensation) and motor-function have not been found , but long-term changes in descending drive and spinal reflexes are thought to trigger secondary adaptive changes to the peripheral neuromusculature (107). This may be exacerbated in patients with low motor-function in whom decreased movement contributes to reduced sensory signaling, although this relation- ship has not been studied.

Changes in cutaneous sensation after stroke may be compounded by those that occur as a function of age. While stroke per se is not age-dependent, the majority of strokes occur after 60 years of age, a period of accelerating decline in both sensory and motor-function. Age-related declines in cutaneous sensation of the hand varied between the fingertips, palm and dorsal regions have been demonstrated(108). The association between changes in motor control and cutaneous sensation with ageing is not strong, and has almost not been tested after stroke.

1.10.1 Cutaneous sensory impairment in the hand post-stroke

Sensory impairments have not been well characterized in chronic stroke patients. The prevalence of impaired cutaneous sensation in a study from Bowden and colleagues (104) was lower than previously reported for patients (109)6 months post-stroke, in

which sensation was categorized as ‘present’, ‘absent’ or ‘impaired’ . Previous reports of greater bilateral impairments in acute and subacute patients were not supported in this study and this may reflect our use of monofilaments, or improvements in contralesional cortical connectivity due to the resolution of diaschisis in the chronic stage post-stroke.

2. HYPOTHESIS

Up to now, various studies had investigated the relationship between the functional status of the descending and ascending pathways and the recovery in stroke patients (75,76,78,79,97,110).

However, most of those, focused on the chronic phase of the stroke, or authors only studied neurophysiological features at one time point and not longitudinally. Moreover, a comprehensive neurophysiological approach to acute stroke patients has not been performed yet. According to the existing literature the presence of a motor evoked potential in the acute phase correlates with the motor recovery and the functional disability at 3 months(75,76,78,97). However, the evolution of the motor potential over time, has not been investigated, as well as if the appearance of a MEP when previously absent is related to a better prognosis. Moreover, no studies as far as we know, investigated if exist a cut-off of neurophysiological parameters which may allow to predict a good recovery in acute stroke patients. The effect of sensory impairment due to stroke, it has been poorly studied and no clear relation has been found.

Due to this premises, our hypothesis are:

1. The presence of a motor evoked potential is a predictor of functional recovery in acute stroke patients as it is its appearance in the early course of the disease.

2. The melioration of MEP features such as amplitude, latency and the central motor conduction time over the course of stroke natural history is related to a better functional prognosis.

3. The sensory impairment is strictly connected to the motor function and therefor related to motor recovery.

3. OBJECTIVES

The purpose of our study is to identify on a neurophysiological approach prognostic factors of stroke recovery.

Specifically the primary objective of our study is:

1. To identify the correlation between neurophysiological features in acute stroke patients measured at different time points and the disability at 3 months

The secondary objectives are:

1. To evaluate the prognostic value of motor performance at baseline on the functional status at 3 months

2. To identify features of the motor evoked potential related to good prognosis

3. To describe the features of sensory impairment in acute stroke patients

4. METHODS

Our research was explained to the participants and all subjects signed the Informed Consent Form (ICF), and the subsequent versions of the study. The procedures used respected the ethical criteria of the Declaration of Helsinki (Fortaleza, Brazil, 2013). Patient identification data are confidential and are not publicly available. Data are protected and participants are informed about the access to their medical history. The identity of the participants is kept confidential: their names, initials, and the numbers assigned to them are not included.

The ethical authorization necessary for the correct development of the project is expressed and approved in the ethical certificate authorized by the Ethical Investigation Committee of HUGTiP on 20/01/2016. We declare no conflict of interest.

4.1 Patients

This study was performed on acute ischemic stroke patients admitted in the stroke unit of the neurology department of HUGTiP between January 2016 – June 2021.

A prospective longitudinal study was designed and consecutive patients were enrolled.

4.2 Inclusion criteria

Patients were included if they fulfilled the following inclusion criteria:

- Age > 18 years

- Functional independence previous to the stroke (mRS <2)

- First ever acute anterior circulation ischemic stroke (anterior cerebral artery, middle cerebral artery, carotid artery either intra or extra cranial)

- Significant impairment of the contra-lesional upper limb defined as NIHSS > 1 in the item 5

- Time since stroke onset of less of 72 hours

4.3 Exclusion criteria

- Contraindication for TMS study (as defined in the introduction section)

- Neurological impairment which may difficult clinical evaluation such as global aphasia, impaired awareness or coma.

- Presence of metal implants, pacemakers or other implantable devices incompatible with TMS pulses delivery.

4.4 Equipment

The study was performed using :

1. A magnetic stimulator MagStim Rapid 2 (Magstim, Withlan UK) able to generate a peak magnetic field of 3,5 Teslas, by delivering byphasic pulses of 400uS of duration.

2. A 10 Channels electromyography equipment (DeltaMed Synergy 10 Channels.) connected by a coaxial cable to the TMS equipment.

3. CMAPs and MEPs were recorded with self-adhesive surface electrodes (Kendall Kittycat foam 4203, Tyco Healthcare) positioned has explained below in the procedure section.

4.5 Procedure

A study dedicated case report form and database were created. Each patient was identified by an aleatory ID .

We recorded:

Demographic (age and sex) and medical history (relevant stroke risk factors, neurologic conditions previous to the stroke, active treatments) data.

Stroke features such as side, severity (measured by NIHSS), presence of arterial occlusion, acute treatment received.

Neuroimaging data (type of neuroimage, size of lesion, area of lesion, if cortical, subcortical or both)

Once included, each patient underwent a thoroughly neurological exploration prior to neurophysiological study that consisted of:

- NIHSS scale

- A complete upper limb muscle strength balance according to the British Medical Council scale (0 = no force generated, 5 = full strength)

- Fugl Meyer Assesment Scale Parts A-D (upper limb) of the paretic arm

- Sensory evaluation of face and limbs including light touch and pain modality

Each session lasted approximately 30 minutes per patient.

4.6 Neurophysiological study

Time from stroke onset to neurophysiological examination was recorded. Each patient was seated in a comfortable chair (if he/she could maintain the seated position, alternatively the exploration was performed in bed).

We used a 10-channels Synergy Electromyography equipment with the possibility of external triggering and a MagStim SuperRapid 2 (MagStim Company , Wales, UK) TMS equipment capable of delivering magnetic pulses of intensities up to 2 Teslas coupled with a figure of 8-shaped (10cm radius) coil.

The study was performed using the same equipment for each session and by the same operator.

All the data were stored in a local hard-drive for offline analysis

4.6.1 Motor study

First, a motor study was performed. As rule of thumb we decided to perform all the exploration bilaterally, always starting from the not affected side.

Upper limb study:

The abductor digiti minimi (ADM) and biceps muscle were identified and two pair of electrodes were positioned in a belly-tendon fashion. The inter electrode (active-reference) distance was at least 2 cm. The reference was preferably situated on a bone or a joint (holecranon for the biceps and fifth metacarpo-phalangeal articulation for the FDI) to minimize movement artefacts.

Motor evoked potential (MEP): a figure of 8-shaped coil was positioned over the area corresponding to the motor representation of the hand (C3 for the left hemisphere and C4 for the right one of the 10-20 EEG system). To identify the hot-spot corresponding to the ADM, slight movements of the coil were performed while delivering TMS pulses at an arbitrary intensity of 70-75% until the site with the higher MEP amplitude was identified.

Given that the study was performed initially on the healthy hemisphere, the resting motor threshold (rMT) was then measured.

We used the Rossini - Rothwell method (111) identifying the minimum stimulator intensity capable of generating 5 out 10 MEPs with an amplitude of at least 50 uV.

The rMT of the healthy hemisphere was used as basal value for the subsequent studies.

After rMT calculation, the stimulator intensity was set to its 120% and 10 single pulses of TMS were delivered. The MEPs generated were recorded from both ADM and biceps muscles at the same time for off-line analysis. If no consistent MEPs were recordable with this intensity, we increased the stimulation up to 150% of rMT. Amplitude, latency and duration were analysed.

The same procedure was then performed on the affected hemisphere. If no MEP was recordable with the highest intensity we tried the supramaximal stimulation of 110% of maximal stimulator output (MSO) (using enhanced mode). If we could not generate any recordable response with this intensity, the MEP was considered absent and the rMT was recorded as 110% of MSO

4.6.2 Cortical silent period (cSP)

To register the silent period we asked the patient to perform a sustained volitional movement consisting in flexion of the elbow and making a fist with the hand contralateral to the stimulated side with the maximum strength possible.

A single TMS pulse at 130% of rMT was then delivered. The cSP was defined as the duration of the absence of electromyographic activity from the stimulus artifact until the appearance of any degree of muscular activity.

Three trials were performed to ensure reproducibility. The mean value of the three trials was calculated. In the affected hand, if no sufficient voluntary contraction could be generated to produce a consistent cSP, the test was not performed.

4.6.3 Central motor conduction time (CMCT):

To study the CMCT we used the ADM as target muscle.

A compound muscle action potential (CMAP) was registered using a conventional electromyography stimulator applied on the ulnar side of the wrist at a distance of approximately 7 cm from the active electrode. A 100 ms square pulse was used with an increasing intensity until a supramaximal stimulation was achieved. Three consistent CMAP were recorded to ensure reproducibility of the recording. Latency and amplitude were recorded.

F Waves of the ADM were recorded delivering 10 stimuli of the same intensity used for the CMAP recording. The minimum latency (Fmin) was used for the CMCT. We then used the MEP latency obtained from the previous motor study to calculate de CMCT bilaterally using the formula: MEP latency - (CMAP latency + Fmin latency - 1 ms) x 0.5.

4.6.4 Facial motor study

As for the upper limb, the study was first performed on the healthy hemisphere. Two electrodes were positioned on the nasalis muscle (active on the belly of the muscle and the reference on the tip of the nose).

A CMAP of the nasalis muscle was registered. An electrical stimulus on the mastoid ipsilateral to the nasalis muscle was applied. A square pulse of 100 ms was used with an increasing intensity until a supramaximal stimulation was achieved. Latency and amplitude were recorded.

MEP: the figure of 8-shaped coil was positioned on the lateral area of the skull (2-3 cm lateral to the area used for the hand) and the intensity of the stimulator was set to the same intensity used for the ADM. Ten stimulus were delivered to ensure for consistency and reproducibility. If no clear MEP was generated, the intensity was increased up to 150% of the rMT. Latency and amplitude were recorded.

To avoid muscular contraction due to diffusion of TMS pulses no further increase of the stimulus intensity was allowed. Given the difficulty to register the MEPs of the face area a slight contraction of the muscle was permitted to facilitate the study.

In the study of the affected side, if no MEP was recorded with the highest stimulation intensity, it was considered absent.

4.7 Sensory study

To evaluate the sensory threshold, a pair of electrodes was applied over the thenar eminence, first over the healthy hand. We chose this site instead of the fingers to avoid the possibility of median nerve entrapment at the wrist or ulnar entrapment at the elbow that could influence the sensory detection.

A squared pulse of 0.5 ms was delivered continuously at a constant frequency of 3 Hz. Patients were asked to subjectively report the first feeling of electricity (sensory threshold) and the first feeling of pain (pain threshold). The measures were repeated 3 times to ensure reproducibility. To avoid habituation a 30 seconds pause was allowed between trials.

If no sensation or pain was noticed at an intensity of 25mA the stimulation went no further. The mean intensity at which the sensory and pain thresholds were detected was calculated and registered.

4.8 7 days / discharge follow – up

After the basal study, a first follow up study was performed during the subacute phase at 7 days from stroke onset. If the patient was discharged before the 7th day, the follow-up study was carried out the last available day.

The follow up study was the same of the basal visit consisting of:

- NIHSS scale
- A complete upper limb muscle strength balance according to the British Medical Council scale (0 = no force generated, 5 = full strength)
- Fugl Meyer Assesment Scale Parts A-D (upper limb) of the paretic arm
- Sensory evaluation of face and limbs including light touch and pain modality
- Furthermore, if available, follow-up neuroimaging data were collected.
- The same neurophysiological assessment was then performed:
  - Upper limb and face bilateral motor study (MEP, rMT, CMAP, CMCT and cSP evaluation)
  - Upper limb sensory and pain thresholds

4.9 3 months evaluation

The final evaluation of the study consisted of a clinical assessment based on the clinical status (NIHSS) and functional disability (mRS) evaluated by a stroke neurologist.

4.10 Statistical analysis

Demographic characteristics were described by frequencies and distribution differences were assessed with the Chi-Square test or Fisher's exact test, as appropriate.

The normality of sample distribution for each variable was assessed by the Kolmogorov-Smirnov Test, assuming normality for a 2-sided sigma < 0.05

Given the presence of values equal to 0 in patients in whom no MEP was evoked, and due to the significance of the presence or absence of the MEP in the statistical analysis, to avoid the influence of such values on the overall measures of the variables, a 1+LN

transformation was performed for proximal and distal MEP latencies. However, for the variables concerning MEP amplitude, a value of 0 was assumed to be equal to the MEP absence, computing in the variable analysis as such.

To evaluate the correlations between stroke features and neurophysiological values at baseline and during follow up we performed pairwise comparisons between groups with the Mann-Whitney U test.

A logistic regression was performed to evaluate the association between patients features and clinical outcome. Due to the high interdependence of neurophysiological variables a univariate ROC curve was performed for each of them, identifying the one with the higher AUC. A logistic regression was then performed to assess the independency of the latter from demographic and clinical variables.

All statistical analyses were performed with the Statistical Package for Social Science (SPSS), IBM version 24.0. A p value <0.05 was considered statistically significant.

5. RESULTS

5.1 Patients recruitment

The study population included 78 patients with first ever anterior ischemic stroke enrolled during the study period.

5.2 Demographic features

Table 1 shows the demographic and clinical characteristics of subjects included.

Table 1. *Demographics and clinical features of study population*

	Total sample
Demographic	N 78
Age (mean ± sd)	64.5 ± 9.4
Gender Male n (%)	49 (62.8)
Hypertension n (%)	44 (56.4)
Diabetes n (%)	25 (32.1)
Hypercholesterolemia n (%)	44 (56.4)
Atrial Fibrillation n (%)	13 (16.7)
COPD n (%)	16 (20.5)
Depression n (%)	8 (10.3)
Clinical Features	
Stroke Side (left) n (%)	45 (57.7)
Arterial Occlusion n (%)	
MCA	21 (26.9)
ACA	0 (0)
TICA	11 (14.1)
ICA	6 (7.7)
No	40 (51.3)
Reperfusion treatment n (%)	
IV tPA	19 (24.4)
EVT	9 (11.5)
IV tPA + TEV	16 (21.5)

No	34 (43.6)
TOAST Classification n (%)	
Aterothrombotic	41 (52.6)
Cardioembolic	10 (12.8)
Lacunar	14 (17.9)
Undetermined	13 (16.7)
Other causes	0 (0)
Stroke Localization n (%)	
Cortical	21 (26.9)
Subcortical	29 (37.2)
Both	28 (35.1)
Basal NIHSS (median ± IQR)	7 [3-17]

COPD: chronic Obstructive Pulmonary Disease; TICA: Terminal Internal Carotid Artery; MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery; ICA Internal carotid artery; tPA: Tissue Plasminogen activator; EVT: Endovascular Treatment; TOAST: Trial of ORG 10172 in acute stroke treatment; NIHSS: National Institute of Health Stroke Scale; .mRS: modified Rankin Score

Of note, patients included were young (mean age 64.5 yo), and were admitted to our hospital with a moderate stroke severity (median NIHSS 7 [3-17]). Stroke topography was evenly distributed among patients being the subcortical localization slightly more frequent (cortical 26.9%, Subcortical 37.2% and both localizations 35.1%). No arterial occlusions were identified during the acute phase in 51.3% of our patients and slightly less than the half received no reperfusion treatments (43.6%). The more frequent stroke etiology was the atherothrombotic (52.6%) followed by lacunar infarcts (17.9%).

5.3 Overall outcome measures at 7 days or discharge

At 7 days or discharge, no deaths were observed during hospitalization. NIHSS at 7 days improved significantly (median 7 days NIHSS 4 [1-7]) with a median mRS of 3 (median mRS 3 [2-4]) at discharge.

5.4 Overall outcome measures at 3 months follow-up

At the 3- months follow-up time point, 2 (2.6%) deaths were recorded. Functional disability improved overall (mRS at 3 -months median 1 [2-3]). No patients were missed at follow-up (Table 2).

Table 2. *Overall baseline clinical outcome*

	Total sample
Outcome measures	*N 78*
NIHSS at 7 days or discharge (median ± IQR)	4 [1-7]
mRS at discharge (median ± IQR)	3 [2-4]
Death during hospitalization n (%)	0 (0)
mRS at 3-month follow-up (median ± IQR)	1 [2-3]
Death at 3-month follow-up n (%)	2 (2.6%)
Lost at 3-month follow- up n (%)	0 (0)

NIHSS: National Institute of Health

5.5 Neurophysiological features and functional outcome

Tables 3, 4 and 5 show neurophysiological data according to functional outcome. Overall, all the measurements involving cortical excitability, MEP features and sensory impairment correlated strongly with functional outcome at 3-months follow-up.

Of note, the presence of the MEP by itself was associated to a good outcome (90.9% vs 13.6% $p < 0.001$) both in the proximal and distal muscle studied. Moreover, MEP amplitude and latency, both in proximal and distal muscles, where associated with good outcome in the basal study (proximal amplitude 0.4uV vs 0uV $p < 0.001$, proximal latency 14.3ms vs 14.5ms p. < 0.001; distal amplitude 0.5uV vs 0uV $p < 0.001$, distal latency 22.5ms vs 22.4ms $p < 0.001$ respectively - Figure 16) and in the day-7 exploration (proximal amplitude 0.85uV vs 0uV $p < 0.001$, proximal latency 15.8ms vs 15ms $p < 0.001$;

distal amplitude 1.1uV vs 0uV p < 0.001, distal latency 22.2 ms vs 22.8ms p < 0.001 respectively). Considering the variation of the MEP features between the 2 study points, a strong statistical correlation was found between proximal and distal MEP features and outcome at 3 months (proximal amplitude 0.3uV vs 0uV p < 0.001, proximal latency -0.3ms vs 0ms p < 0.001; distal amplitude 0.3uV vs 0uV p < 0.001, distal latency -0.5 ms vs 0ms p < 0.001 respectively). Finally, the appearance of the MEP at day 7 in patients who did not present it at the basal study correlated to a better mRS at 3-months (median, 4 vs 0 p < 0.001).

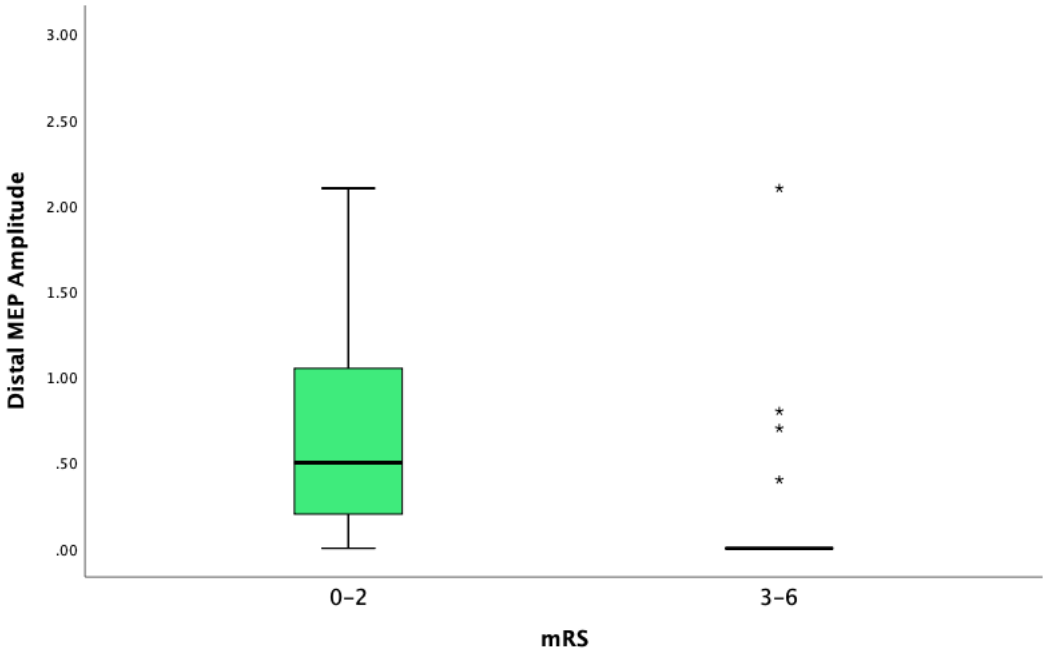

Figure 16. *Distal MEP amplitude (mV) according to mRS at 3 months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value*

Table 3. *Basal Neurophysiologic and clinical features and functional impairment at 3-month follow up*

Neurophysiologic Features at basal study	*mRS ≤ 2 at 3-month follow up n=55*	*mRS > 2 at 3-month follow up n= 23*	*p*
rMT (median ± IQR)	82 [75-89]	110 [110-110]	<0.001
Proximal MEP presence n (%)	50 (90.9)	3 (13.6)	<0.001
Distal MEP presence n (%)	50 (90.9)	3 (13.6)	<0.001
cSP (median ± IQR)	92 [0-124]	0 [0-0]	<0.001
Proximal MEP amplitude (median ± IQR)	0.4 [0.2-0.7]	0 [0-0]	<0.001
Proximal MEP latency* (median ± IQR)	14.3 [14.2-15.6]	14.5 [13.8 -15]	<0.001
Distal MEP amplitude (median ± IQR)	0.5 [0.2-1.1]	0 [0-0]	<0.001
Distal MEP latency* (median ± IQR)	22.5 [21.5-23]	22.4 [19.7-24.2]	<0.001
Facial MEP amplitude (median ± IQR)	0.1 [0-0.2]	0 [0-0]	0.008
Facial MEP latency (median ± IQR)	14.2 [13.7-14.7]	13.8 [13.5-14.3]	0.407
CMCT (median ± IQR)	7.5 [6.5-8]	6.5 [6.3-7]	0.728
Sensory Threshold (median ± IQR)	14 [8-20]	19 [16-25]	0.006
Pain Threshold (median ± IQR)	25 [17-25]	25 [25-25]	0.003
Clinical Features			
Fugl Mayer scale (A+B+C items) (median ± IQR)	26 [13-38]	13 [8-16]	<0.001
BMC strength (median ± IQR)	31 [24-33]	12 [8-18]	<0.001

rMT: resting motor threshold (MSO%); MEP: motor evoked potential (mV); cSP: cortical silent period (ms); CMCT: central motor conduction time(ms)*Natural Logarithmic transformation performed

Cortical excitability measured by the rMT was significantly lower in patients with functional independence compared to the patients dependent at 3 months (rMT median 82% vs 110% $p < 0.001$) . Again, a decrease of the excitability between the basal and the day-7 test was found to be associated significantly with good functional outcome (median rMT -2% vs 0% $p < 0.001$) as shown in figure 17. The contralateral silent period duration, as other indirect measure of cortical excitability, was again found to be strongly associated to a better functional outcome at basal and day-7 exploration as well as its

variation between those 2 time points (cSP median 92ms vs 0ms p < 0.001, 111ms vs 0ms p < 0.001 and 6ms vs0ms p < 0.001 respectively).

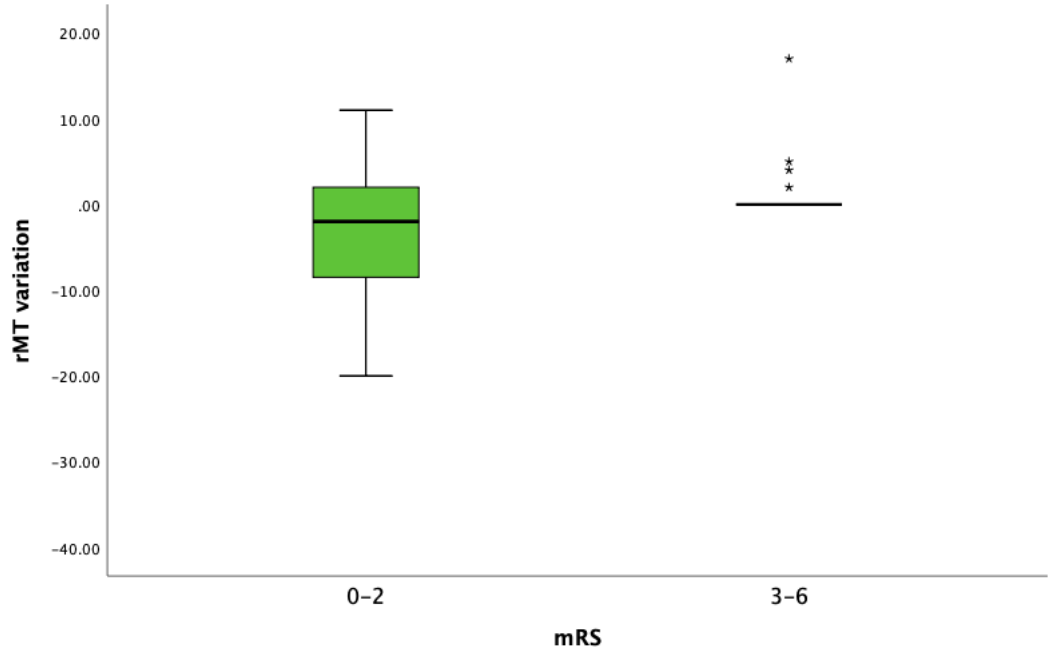

Figure 17. *rMT (%MSO)variation between basal and day 7 study according to mRS at 3-months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value*

Sensory impairment measured by the sensory and pain perception threshold was related significantly to the functional outcome both at basal and day-7 studies (basal sensory median 14mA vs 19mA p= 0.006, pain 25mA vs 25mA p= 0.003; day-7 sensory 12mA vs 19mA p < 0.001, pain 16.5mA vs 25mA p < 0.001) . Once more, the differences of sensory and pain threshold between basal and day-7 were significantly related to a better functional outcome (sensory median -2mA vs -1mA p= 0.001, pain median -4mA vs 0mA p < 0.001 – Figure 18 -19)

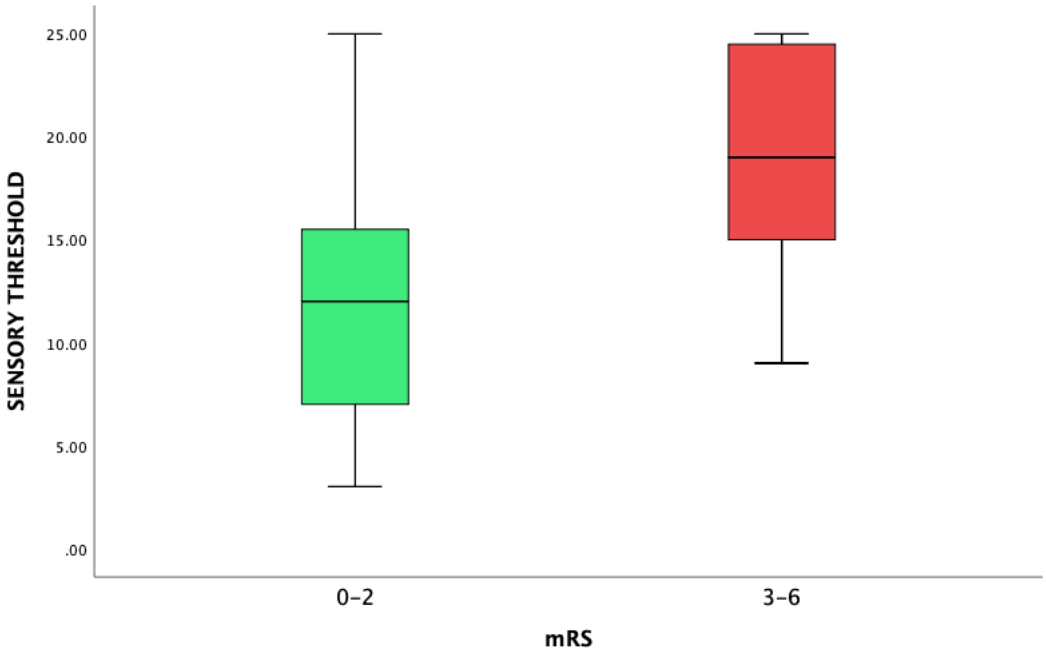

Figure 18. *Basal sensory threshold (mV) according to functional outcome at 3-months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value*

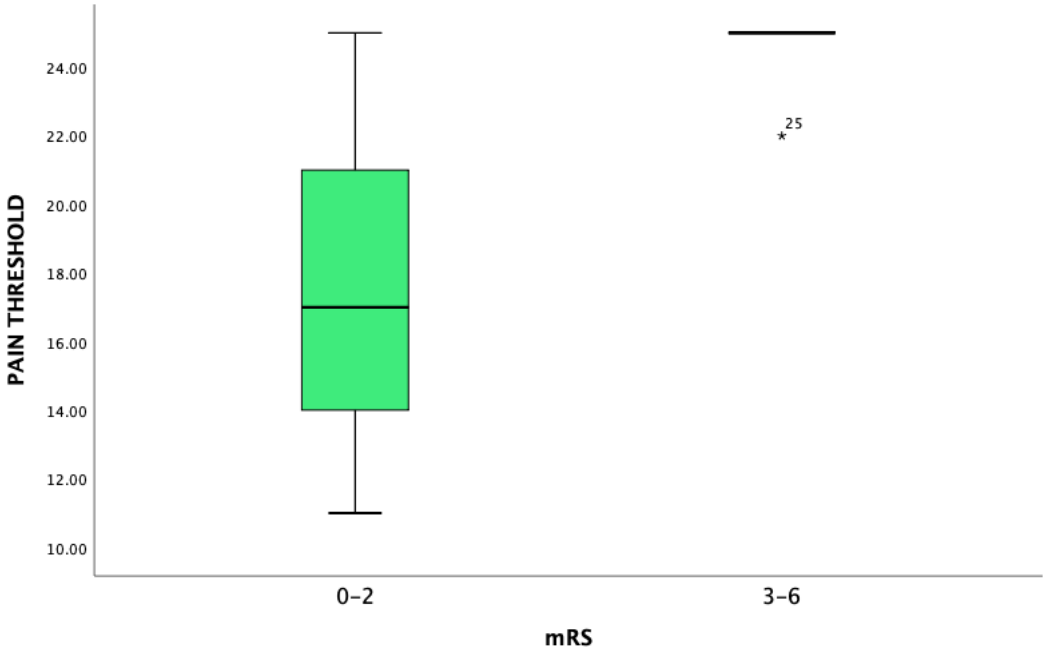

Figure 19. *Basal pain threshold (mV) according to functional outcome at 3-months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value.*

Indeed an higher BMC and Fugl-Meyer punctuation both at basal (31 vs 12 and 26 vs 13 $p < 0.001$ respectively) and day-7 (31 vs 14 and 31 vs 13 $p < 0.001$ respectively) where associated to a better functional outcome at 3 months as shown in Figure 20 and 21.

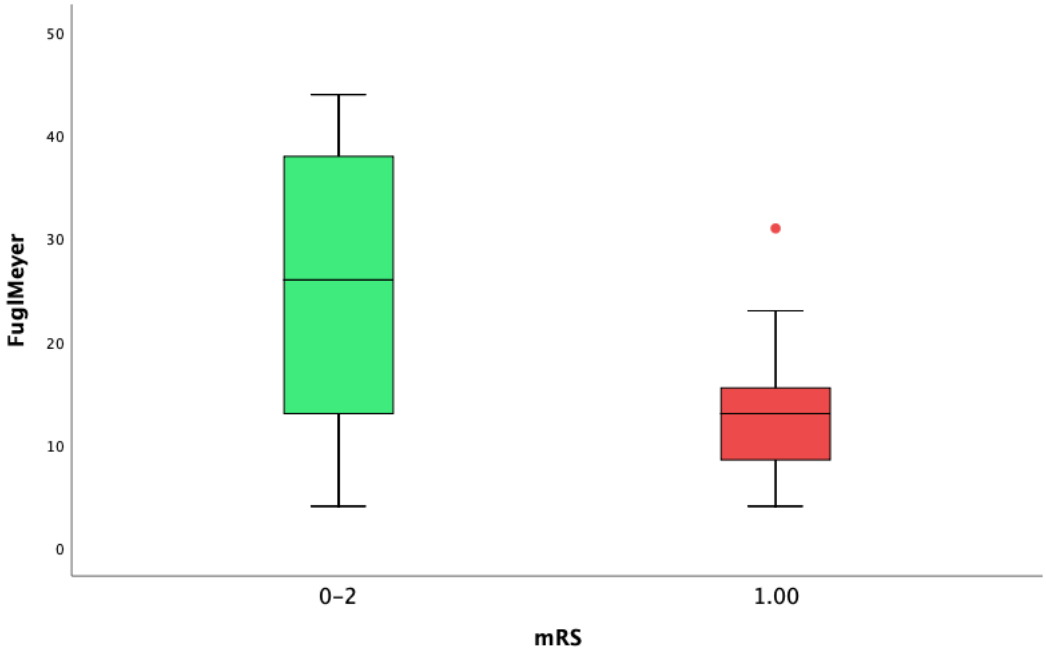

Figure 20. *Basal FuglMeyer scale according to mRS at 3 –months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value*.

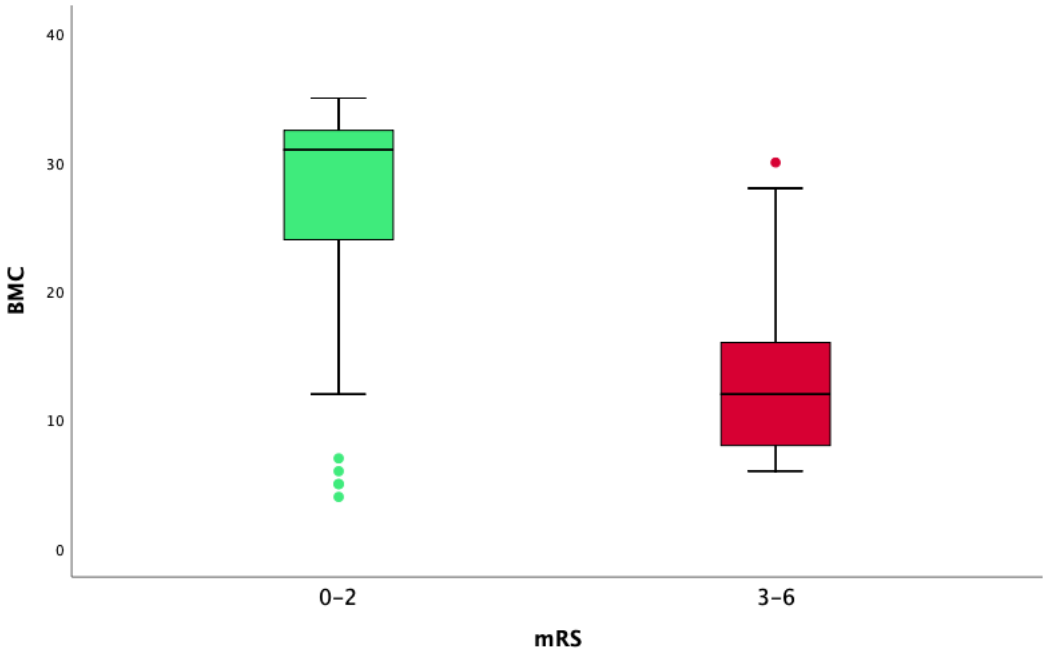

Figure 21. *Basal BMC score according to mRS at 3 –months. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value*

Table 4. *7 days Neurophysiologic and clinical features by functional impairment at 3-month follow up*

Neurophysiologic Features at 7 days or discharge	mRS ≤ 2 at 3-month follow up n = 55	mRS > 2 at 3-month follow up n= 23	p
rMT (mean ± sd)	75 [69-85]	110 [110-110]	< 0.001
Proximal MEP presence n (%)	54 (98.2)	2 (9.1)	< 0.001
Distal MEP presence n (%)	51 (91.7)	3 (13.6)	< 0.001
cSP (median ± IQR)	111 [0-176]	0 [0-0]	< 0.001
Proximal MEP amplitude (median ± IQR)	0.85 [0-4 -1.2]	0 [0-0]	< 0.001
Proximal MEP latency* (median ± IQR)	13.8 [13.5-14.4]	15 [14.7-15.1]	< 0.001
Distal MEP amplitude (median ± IQR)	1.1 [0.8 -1.5]	0 [0-0]	< 0.001
Distal MEP latency* (median ± IQR)	22.2 [21.2-22.6]	22.8 [21.2-24.5]	< 0.001
Facial MEP amplitude (median ± IQR)	0.1 [0 – 0.3]	0 [0-0]	0.007
Facial MEP latency (median ± IQR)	13.9 [13.5-14.7]	13.9 [13.2-14.2]	0.730
MEP appeareance at 7 days (n%)	4	0	< 0.001
CMCT (median ± IQR)	7.4[6.5-7.9]	6.1 [5.5-6.6]	< 0.001
Sensory Threshold (median ± IQR)	12 [7-15]	19 [15-25]	< 0.001
Pain Threshold (median ± IQR)	16.5 [14-21]	25 [25-25]	< 0.001
Clinical Features			< 0.001
Fugl Mayer scale (A+B+C items) (median ± IQR)	31 [21.7-29]	14 [10-17]	< 0.001
BMC strength (median ± IQR)	31 [27-33]	13 [10-16]	< 0.001

rMT: resting motor threshold (%MSO) ; MEP: motor evoked potential (mV); cSP: cortical silent period (ms); CMCT: central motor conduction time(ms)

*Natural Logarithmic transformation performed

Table 5. *Variation between basal and day 7 studies of neurophysiologic and clinical features by functional impairment at 3-month follow up*

Neurophysiologic Features Variation Between day 7 and basal	mRS ≤ 2 at 3-month follow up n = 55	mRS > 2 at 3-month follow up n= 23	p
rMT (median ± IQR)	-2 [-9 - 2]	0 [0-0]	<0.001
cSP (median ± IQR)	111 [0-176]	0 [0-0]	0.001
Proximal MEP amplitude (median ± IQR)	0.3 [0 – 0.7]	0 [0-0]	<0.001
Proximal MEP latency* (median ± IQR)	- 0.3 [- 0.6 - -0.05]	0 [0-0]	<0.001
Distal MEP amplitude (median ± IQR)	0.3 [- 0.1 - -0.5]	0 [0-0]	0.001
Distal MEP latency* (median ± IQR)	- 0.5 [-0.3 - 0.7]	0.0	<0.001
MEP appeareance at 7 days (n%)	4 (7)	0 (0)	<0.001
CMCT (median ± IQR)	0 [-0.25 – 1.5]	0 [0-0]	0.580
Sensory Threshold (median ± IQR)	-2 [-4.5 - -1]	-1 [0-1]	0.001
Pain Threshold (median ± IQR)	-4 [-7- -1]	0 [0-0]	<0.001
Clinical Features			<0.001
Fugl Mayer scale (A+B+C items) (median ± IQR)	1 [5-10]	0 [-2 - 2]	<0.001
BMC strength (median ± IQR)	1 [0-3]	1 [-1 – 2.5]	0.266

rMT: resting motor threshold (%MSO); MEP: motor evoked potential (mV); cSP: cortical silent period (cSP); CMCT: central motor conduction time(ms)

*Natural Logarithmic transformation performed

5.6 Effects of acute stroke on neurophysiological features

We then studied the changes of a series of neurophysiological variables according to the affected or unaffected brain side as shown in Tables 6 and 7.

Table 6. *Basal neurophysiologic and clinical features of study population according to lesion side.*

Neurophysiologic Features at basal study	Affected Hemisphere N 78	Unaffected hemisphere N 78	p
rMT (median ± IQR)	86.5 [76.5-110]	62 [60-64]	<0.001
Proximal MEP presence n (%)	54 (69.2)	78 (100)	
Distal MEP presence n (%)	54 (69.2)	78 (100)	
cSP (median ± IQR)	74 [0-109]	189 [165-211]	<0.001
Proximal MEP amplitude (median ± IQR)	0.3 [0-0.6]	0.9 [0.7-1.1]	<0.001
Proximal MEP latency* (median ± IQR)	14 [14.7-15.2]	14.1 [13.4-14.6]	0.020
Distal MEP amplitude (median ± IQR)	0.35 [0-0.32]	1.9 [1.6-2.4]	<0.001
Distal MEP latency* (median ± IQR)	22.6 [21.6-22.7]	22 [21.2-23.2]	<0.001
CMCT (median ± IQR)	7.5 [6.6-8.1]	7.1 [6.5 – 7.6]	<0.001
Sensory Threshold (median ± IQR)	17 [11.2-22.2]	4 [3-5]	<0.001
Pain Threshold (median ± IQR)	25 [20-25]	9 [8-10]	<0.001
Clinical Features			
Fugl Mayer scale (A+B+C items) (median ± IQR)	21 [12-30]		
BMC strength (median ± IQR)	27 [12-32]		

rMT: resting motor threshold (%MSO); MEP: Motor Evoked Potential (mV); cSP contralateral silent period (ms); CMCT: central motor conduction Time(ms); BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA. *Natural Logarithmic transformation performed

As a matter of facts, the cortical excitability was lower both in its facilitatory neurons (rMT 84% vs 62% $p < 0.001$) as well as the inhibitory ones (cSP 60 vs 214 $p < 0.001$) both at basal and a day-7 studies. Proximal MEP amplitude reduction and latency prolongation were both observed in the affected side compared to the healthy one. The

same observation could be inferred about the distal MEP at both time points. Interestingly a slight but significant prolongation of the CMCT was observed in the affected side (7.5ms vs 7.2ms p 0.01).

Moreover, both sensory and pain perceptions were found to be significantly altered by the stroke at basal (sensory 17mA vs 4mA, pain 25mA vs 9 mA p <0.001) and day-7 (sensory 14mA vs 4mA, pain 19mA vs 9 mA p < 0.001) .

Table 7. *Neurophysiologic and clinical features of study population at 7 days or discharge according to lesion side.*

Neurophysiologic Features	Affected Hemisphere N 78	Unaffected hemisphere N 78	p
rMT (median ± IQR)	84 [62-110]	62 [59-63]	<0.001
Proximal MEP presence n (%)	57 (73.1)	78 (100)	
Distal MEP presence n (%)	55 (70.5)	78 (100)	
cSP (median ± IQR)	60 [0-144]	214 [114-246]	<0.001
Proximal MEP amplitude (median ± IQR)	0.5 [0-1.1]	0.85 [0.4-1.1]	0.039
Proximal MEP latency* (median ± IQR)	14 [13.6-14.6]	13.9 [13.5-14.3]	0.265
Distal MEP amplitude (median ± IQR)	0.9 [0-1.4]	2 [1.7-2.3]	<0.001
Distal MEP latency* (median ± IQR)	22.2 [21.3-23.2]	21.7 [21.1-21.9]	<0.001
CMCT(median ± IQR)	7.5 [6.7-7.9]	7.2 [6.4 – 7.6]	0.001
Sensory Threshold (median ± IQR)	14 [9-18.5]	4 [3-4]	<0.001
Pain Threshold (median ± IQR)	19 [14.5-25]	9 [8-10]	<0.001
Clinical Features			
Fugl Mayer scale (A+B+C items) (median ± IQR)	25 [16.5-37]		
BMC strength (median ± IQR)	28 [15.5-33]		

rMT: resting motor threshold (%MSO); MEP: Motor Evoked Potential (mV); cSP contralateral silent period (ms); CMCT: central motor conduction Time(ms); BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA.
*Natural Logarithmic transformation performed

5.7 Patients clinical and neurophysiological features according to MEP presence

Tables 8 to 11 show clinical and neurophysiological features according to proximal and distal MEP presence at basal and 7 day studies.

Table 8. *Clinical features of study population according to proximal MEP presence or absence at basal study*

	Proximal MEP Basal		
	Proximal MEP absent N 24	**Proximal MEP present N 54**	**p**
Basal NIHSS (median ± IQR)	10 [6-19]	7 [2-15]	<0.001
NIHSS at 7 days or discharge (median ±IQR)	7 [3-12]	3 [1-5]	0.001
mRS at discharge (median ± IQR)	4 [2-4]	2 [2-3]	<0.001
mRS at 3-month follow-up (median ± IQR)	3 [3-4]	1 [0-2]	<0.001
Fugl-Meyer scale (median ± IQR)	12 [8-20]	26 [13-38]	<0.001
BMC scale (median ± IQR)	11 [7-17]	31 [23-33]	<0.001
Side Left n (%)	13 (54)	32 (59.3)	0.676
Localization			0.001
Cortical	3 (12.5)	18 (33.3)	
Subcortical	5 (20.5)	24 (44.4)	
Both	16 (66.7)	12 (22.2)	
Arterial Occlusion n (%)			0.008
MCA	9 (37.5)	12 (22.2)	
ACA	0 (0)	0 (0)	
TICA	4 (16.7)	7 (13)	
ICA	4 (16.7)	2 (3.7)	
No	7 (29.2)	33 (61.1)	
Reperfusion treatment n (%)			0.031
		11 (20.4)	
IV tPA	8 (33.3)	4 (7.4)	
EVT	5 (20.8)	10 (18.5)	
IV tPA + TEV	5 (20.8)	29 (53.7)	
No	9 (37.5)		
TOAST Classification n (%)			0.671
Aterothrombotic	14 (58.3)	27 (50)	
Cardioembolic	4 (16.7)	6 (11.1)	
Lacunar	0 (0)	14 (25.9)	
Undetermined	6 (25)	7 (13)	
Other causes	0 (0)	0 (0)	

rMT: resting motor threshold (%MSO); MEP: Motor Evoked Potential (mV); cSP contralateral silent period(ms); CMCT: central motor conduction Time(ms); BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA. TICA:Terminal Internal Carotid Artery; MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery; ICA Internal carotid artery; tPA: Tissue Plasminogen activator; EVT: Endovascular Treatment; TOAST: Trial of ORG 10172 in acute stroke treatment; NIHSS: National Institute of Health Stroke Scale; mRS: modified Rankin Score

Among the clinical features, MEP presence was related to a lower NIHSS at admission (p <0.001), 7 days (0.001) and both the clinical functional scales (BMC and Fugl-Meyer p < 0.001) as shown in Figure 22. All those values were found to be statistically significant at the two time points, both for the proximal and the distal MEP. No lesion side difference was found (p > 0.05) for any of the time points of the MEP.

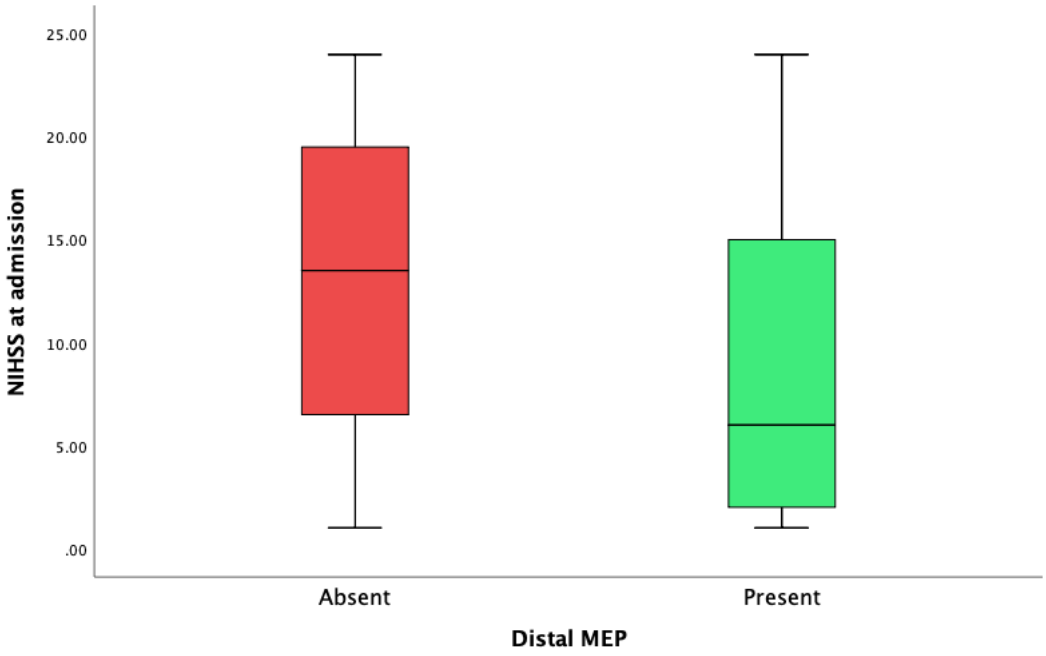

Figure 22. *NIHSS at admission according to MEP presence. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value.*

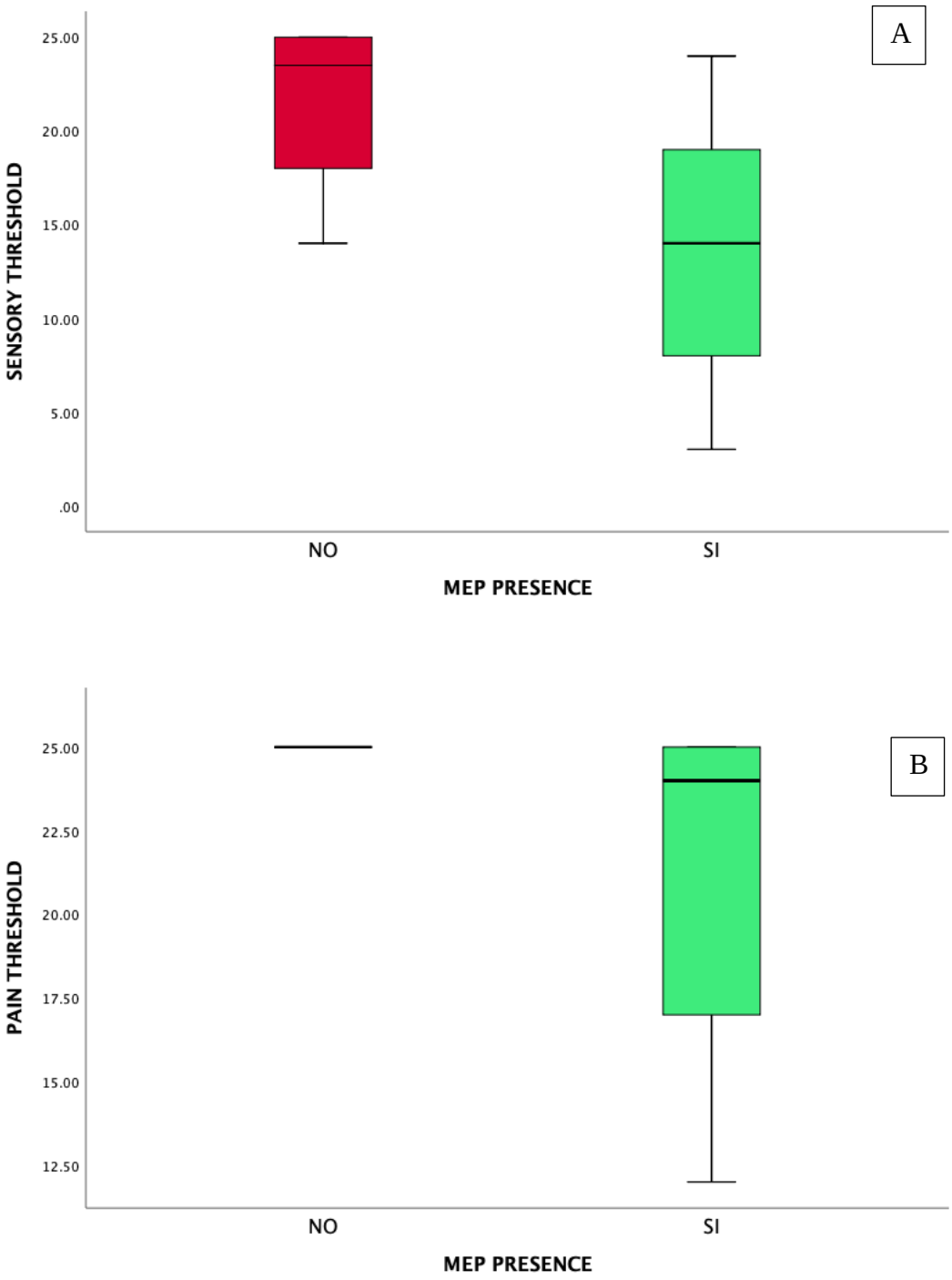

Figure 23. *Sensory (A) and Pain (B) thresholds according to MEP presence. Boxplots show percentiles 25 -75, outer lines show maximum and minimum value. Bolded line represents median value.*

Table 9. *Clinical features of study population according to distal MEP presence or absence at basal study*

	Distal MEP Basal		
	Distal MEP Absent N 24	**Distal MEP present N 54**	**p**
Basal NIHSS (median ± IQR)	13 [6-19]	6 [2-15]	<0.001
NIHSS at 7 days or discharge (median ± IQR)	7 [5-13]	3 [1-5]	<0.001
mRS at discharge (median ± IQR)	4 [4-4]	2 [2-3]	<0.001
mRS at 3-month follow-up (median ± IQR)	3 [3-4]	1 [0-2]	<0.001
Fugl-Meyer scale (median ± IQR)	13 [8-23]	25 [13-37]	<0.001
BMC scale (median ± IQR)	12 [7-25]	31 [21-31]	<0.001
Localization			0.008
Cortical	3 (12.5)	18 (33.3)	
Subcortical	5 (20.8)	24 (44.4)	
Both	16 (16.7)	12 (22.2)	
Arterial Occlusion n (%)			0.008
	9 (37.5)	12 (22.2)	
MCA	0 (0)	0 (0)	
ACA	4 (16.7)	7 (13)	
TICA	4 (16.7)	2 (3.7)	
ICA	7 (29.2)	33 (61.1)	
No			
Reperfusion treatment n (%)			0.031
IV tPA	8 (33.3)	11 (20.4)	
EVT	5 (20.8)	4 (7.4)	
IV tPA + TEV	5 (20.8)	10 (18.5)	
No	9 (37.5)	29 (53.7)	
TOAST Classification n (%)			0.671
Aterothrombotic	14 (58.3)	27 (50)	
Cardioembolic	4 (16.7)	6 (11.1)	
Lacunar	0 (0)	14 (25.9)	
Undetermined	6 (25)	7 (13)	
Other causes	0 (0)	0 (0)	

rMT: resting motor threshold; MEP: Motor Evoked Potential; cSP contralateral silent period; CMCT: central motor conduction Time; BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA. TICA:Terminal Internal Carotid Artery; MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery; ICA Internal carotid artery; tPA: Tissue Plasminogen activator; EVT: Endovascular Treatment; TOAST: Trial of ORG 10172 in acute stroke treatment; NIHSS: National Institute of Health Stroke Scale; .mRS: modified Rankin Score

Topographically, larger stroke due to arterial occlusion of any kind was found to be related to MEP presence both with the proximal and the distal one (p 0.008 at basal and p 0.044 at day 7) as well as the involvement of both the cortical and subcortical infarction (p 0.001 basally and p 0.008 at day 7). No significant relation was found between the stroke etiology and MEP presence.

Table 10. *Clinical features of study population according to proximal MEP presence or absence at 7 days study*

	7 days Proximal MEP		
	Proximal MEP absent N 23	**Proximal MEP present N 55**	**p**
Basal NIHSS (median ± IQR)	15 [8-19]	6 [2-15]	<0.001
NIHSS at 7 days or discharge (median ± IQR)	8 [5-13]	3 [1-5]	<0.001
mRS at discharge (median ± IQR)	4 [4-4]	2 [2-3]	<0.001
mRS at 3-month follow-up (median ± IQR)	3 [3-4]	1 [0-2]	<0.001
Fugl-Meyer scale (median ± IQR)	13 [8-16]	26 [13-38]	<0.001
BMC scale (median ± IQR)	12 [8-18]	31 [24-33]	<0.001
Left side n (%)	12 (52)	33 (60)	0.526
Localization			<0.001
Cortical	3 (13)	18(32.7)	
Subcortical	4 (17.4)	25 (45.5)	
Both	16 (69.6)	12 (21.8)	
Arterial Occlusion n (%)			0.031
MCA	10 (43.5)	11 (20)	
ACA	0 (0)	0 (0)	
TICA	2 (8.7)	9 (16.4)	
ICA	4 (17.4)	2 (3.6)	
No	7 (30.4)	33 (60)	
Reperfusion treatment n (%)			0.070
IV tPA	8 (34.8)	11 (20)	
EVT	5 (21.7)	4 (7.3)	
IV tPA + TEV	4 (17.4)	11 (20)	
No	5 (21.7)	29 (52.7)	
TOAST Classification n (%)			0.834
Aterothrombotic	13 (56.5)	28 (50.9)	
Cardioembolic	4 (17.4)	6 (10.9)	
Lacunar	0 (0)	14 (25.5)	
Undetermined	6 (26.1)	7 (12.7)	
Other causes	0 (0)	0 (0)	

rMT: resting motor threshold; MEP: Motor Evoked Potential; cSP contralateral silent period; CMCT: central motor conduction Time; BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA.
TICA:Terminal Internal Carotid Artery; MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery; ICA Internal carotid artery; tPA: Tissue Plasminogen activator; EVT: Endovascular Treatment; TOAST: Trial of ORG 10172 in acute stroke treatment; NIHSS: National Institute of Health Stroke Scale; .mRS: modified Rankin Score

Table 11. *Clinical features of study population according to distal MEP presence or absence at 7 days study*

	7 days Distal MEP		
	Distal MEP absent N 21	Distal MEP present N 57	p
Basal NIHSS (median ± IQR)	15 [7-19]	6 [2-15]	<0.001
NIHSS at 7 days or discharge (median ± IQR)	7 [5-13]	3 [1-5]	<0.001
mRS at discharge (median ± IQR)	4 [4-4]	2 [2-3]	<0.001
mRS at 3-month follow-up (median ± IQR)	3 [3-4]	1[0-2]	<0.001
Fugl-Meyer scale (median ± IQR)	12 [8-16]	26 [13-38]	<0.001
BMC scale (median ± IQR)	12 [8-18]	31 [24-33]	<0.001
Left side n (%)	9 (42.9)	36 (63.2)	0.111
Localization			0.008
Cortical	3 (14.3)	18 (31.6)	
Subcortical	5 (23.8)	24 (42.1)	
Both	13 (61.9)	15 (26.3)	
Arterial Occlusion n (%)			0.044
MCA	10 (47.6)	11 (19.3)	
ACA	0 (0)	0 (0)	
TICA	2 (9.5)	9 (15.8)	
ICA	3 (14.3)	3 (5.3)	
No	6 (28.6)	34 (59.6)	
Reperfusion treatment n (%)			0.108
IV tPA	8 (38.1)	11 (19.3)	
EVT	4 (19)	5 (8.8)	
IV tPA + TEV	4 (19)	11 (19.3)	
No	5 (23.8)	29 (50.9)	
TOAST Classification n (%)			0.387
Aterothrombotic	10 (47.6)	31 (54.4)	
Cardioembolic	4 (19)	6 (10.5)	
Lacunar	0 (0)	14 (24.6)	
Undetermined	7 (33.3)	6 (10.5)	
Other causes	0 (0)	0 (0)	

rMT: resting motor threshold; MEP: Motor Evoked Potential; cSP contralateral silent period; CMCT: central motor conduction Time; BMC: British medical council strength scale: rMT is shown as % of maximum stimulator output. MEP amplitude is expressed in mV; MEP Latency, cSP and CMCT are expressed in ms ; Sensory and pain threshold are expressed in mA. TICA:Terminal Internal Carotid Artery; MCA: Middle Cerebral Artery; ACA: Anterior Cerebral Artery; ICA Internal carotid artery; tPA: Tissue Plasminogen activator; EVT: Endovascular Treatment; TOAST: Trial of ORG 10172 in acute stroke treatment; NIHSS: National Institute of Health Stroke Scale; .mRS: modified Rankin Score

5.8 Univariate analysis of Receiver operating characteristic curve and multivariate analysis

Given the results of the univariate analysis, and the known strong interdependence of several of the neurophysiological parameters studied, we performed a ROC analysis of each of them with the aim of identifying the better AUC. Table 12 shows the results for basal and day 7.

The better AUC was found to be related to the distal MEP amplitude at basal study (AUC 0.916) with a cut-off point of 0.5mA (Sensitivity 74%, Specificity 100%).

Table 12. *Area under the curve for the ROC analysis for each of the studied variables.*

	AUC	
Item	Basal	Day 7
rMT	0.340	0.082
Proximal MEP Latency	0.191	0.192
Proximal MEP Amplitude	0.911	0.865
Distal MEP Latency	0.259	0.259
Distal MEP Amplitude	0.916	0.877
CMCT	0.543	0.523
CSP	0.742	0.760
Sensory Threshold	0.303	0.182
Pain Threshold	0.317	0.069

rMT: resting motor threshold (%MSO) ; MEP: motor evoked potential (mV): CMCT: central motor conduction time (ms) : cSP: cortical silent period (ms)

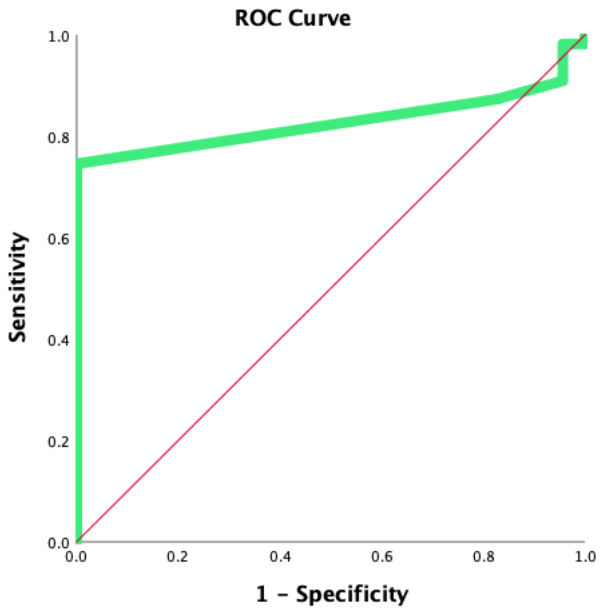

Figure 24. *ROC curve of MEP amplitude and mRS 0-2 at 3 months*

Then, a logistic regression model adjusted for demographic features such as age and gender, clinical variables (NIHSS at admission, stroke localization, etiology and reperfusion treatment) and the distal MEP amplitude at basal study as specified above, showed that the latter was independently associated to a good outcome at 3-monts (B -0.013 95% CI -0.002 – 0.100, p <0.001) – (Table 13).

Table 13. *Logistic regression model. Multivariate analysis of factor associated to a good outcome in acute stroke patients.*

	B	95% CI	p
Age	0.950	0.868 – 1.041	0.274
Gender	0.695	0.100 – 4.825	0.713
Occlusion	1.346	0.680– 2.662	0.394
Reperfusion	0.376	0.134—1.051	0.062
Localization	1.486	0.471 – 4.689	0.499
Etiology	1.720	0.738 – 4.009	0.209
Basal NIHSS	1.129	0.974 – 1.1310	0.108
Distal MEP amplitude	0.013	0.002 – 0.100	<0.001

NIHSS: National Institute of Health Stroke Scale; MEP: motor evoked potential (mV)

6. DISCUSSION

Our study investigated the effects of acute ischemic stroke on several neurophysiological measures.

6.1 Objective 1. To identify the correlation between neurophysiological features in acute stroke patients measured at different time points and the disability at 3 months

According to our data, the relation between the physiological status of the motor area and the functional disability at 3-months is straightforward.
Motoneuron function both facilitatory and inhibitory, did show a profound alteration both at basal and day 7 tests. The increase of the rMT reflects the loss of motoneurons due to the acute stroke, as well as the stunned proportion of the motoneurons in the penumbral area of the infarcts. According to this, the higher the rMT, the greater the impairment of the motor function, with a direct translation into the motor function. Interestingly, when no variation of rMT was found between the 2 time points (table 5), no clear improvement in motor function was found, reflecting the probable established loss of motor neurons.

On the other side, the inhibitory circuits at motor cortex identified by the cSP, showed, as well as the facilitator neurons, a great alteration following acute stroke(83–85,87). The absence of cSP in our group was both consequence of the impossibility to generate volitional movements with sufficient strength and indeed, the loss of inhibitory neurons at cortical level. However, although it is not possible to distinguish those two mechanisms, in patients with a BMC scale adequate, cSP was still significantly lower than the one generated stimulating the unaffected side. Previously published classical studies showed both a prolonged and a shortened cSP in stroke patients, although those studies were performed mostly in the chronic phase(70,87) and their results correlated to a worse prognosis. What we found could be related to the fact that during the hyperacute phase, the inhibitory circuits of the motor cortex were not able to function properly, not being able to interrupt a volitional contraction adequately. Again, an increase of cSP duration was observed in patients with a good prognosis,

indicating an ongoing restoration of the inhibitory cortical function.
Both cortical motoneuron roles, have already been associated to the motor function recovery in stroke patients. However, we demonstrated that, not only the changes in the acute phase are significant as predictors of good outcome, but, once the plasticity mechanisms start to take place, the changes in cortical excitability still demonstrate a good predictive value on motor outcome.

A part from excitability, the integrity of the pyramidal tract is essential for motor function. Our data confirm previous observations that the MEP presence correlates strongly with functional outcome(92,93,97). We included both a proximal and a distal muscle MEP because, as already known, motor recovery in stroke patient might begin asymmetrically, starting proximally or distally according to lesion site(100). However, unfortunately, in our group of patients, when distal MEP was absent, the proximal was mostly absent as well, so no clear inferences could be made.

On MEP features, we went further exploring each of its features at admission, and day 7 or discharge. Indeed, patients whom presented an higher amplitude, indicating a more preserved motoneuron pool(18), and a shorter latency, which in turn expressed both an higher synchronization of the motoneurons activation(46) and a better state of the pyramidal tract, showed a better functional outcome at 3-moths. Interestingly, as observed with the cortical excitability measures, the absence of bettering during the 2 studies, was associated to a worse outcome. Previous literature already showed a strong correlation of MEP and motor function(72,76,77,79,81). However, we prospectively studied the behavior of latency and amplitude during the acute phase, again manifesting that, as sooner the cortical and pyramidal function improves or is restored, the better the outcome will be.

We then investigated the effects of acute stroke on the lesional hemisphere. Almost 30% of patients did not have an evocable MEP at basal study neither the proximal nor the distal muscle, as previously reported by Escudero et al (76).
Again, all the measures of cortical function (rMT, cSP) and of pyramidal tract integrity (CMCT, MEP features when present) showed a profound alteration. Although the impairment was found to be mostly of axonal nature (MEP amplitude decrease), both MEP latency and CMCT were found to be prolonged on the affected side, reflecting

both the effects of axonal loss (or no excitability of motor neurons) and the disruption of potential transmission over a lesioned pyramidal tract (74). Of note, a slight increase of the CMCT was observed, confirming a previous report by Heald et al.

According to our statistical methodology, we identified the MEP amplitude at basal tests as the stronger predictor of good recovery at 3 months. This remained valid even after correction for known confounders. It is already known from previous literature that the presence of MEP is related to a good prognosis. However, here we identified the amplitude as the better predictor of motor function at 3-months, independently.
Of note, compared to previous studies, we did include patients with a moderate motor impairment, disregarding of the rest of the neurological impairment. As we could see, even if the movement is preserved even slightly, the MEP is not unequivocally evocable. We think that this might be due to a shared role of cortical excitability and CST integrity in MEP generation. This is supported by the fact that the lesional topography varied significantly among patients according to MEP presence (table 7-10). Most of MEP present patients suffered a stroke which involved both cortical and subcortical areas, disrupting the motor cortex and the CST, while on the contrary, most of the patients who still presented an evocable MEP suffered a subcortical stroke, probably preserving motor cortex, and activating alternative pathways, as reflected by the significant increase in lacunar etiology in the latter group. Indeed, it is known that MEPs recorded from patients with superficial (cortical) infarcts have smaller amplitudes and longer CMCTs than those in deep infarcts, in correlation with worse prognosis in the cortical infarct group (71). We can infer that a severe stroke with a lesion involving both cortical and subcortical areas provokes changes in the motor pathways more severe and with less plastic reserve available to ameliorate stroke prognosis.

To finalize, the appearance of an MEP (indistinctively proximal or distal) during the first 7 days or before discharge was significantly related to a better prognosis. Three out of the 4 patients who presented a MEP at 7 days, suffered a subcortical restricted stroke. Again, we do suggest that the preservation of the cortex, facilitate cortical plasticity and in turn the generation of alternative pathways, as fast as 7 days. Another valuable option could be that the motor cortex could be “stunned” by the deep region stroke

and although the CST is not affected, the excitability of the motoneurons is, preventing the generation of a valid potential. After few days, this alteration ameliorates, producing a MEP as observed.

In conclusion, as predicted, both cortical excitability and corticospinal tract involvement are directly and strongly related to functional outcome at 3 months. No clear differences have been found among proximal and distal target muscle. The appearance of a MEP when previously not present is an indicator of good recovery.

6.2 Objective 2.1. To evaluate the influence of motor performance at baseline on the functional status at 3 months

As expected, motor performance in both the muscular strength and the dexterity and performance based index, correlated to a good outcome. Again, the MEP presence was found to be related to a better motor performance as already described, so our data confirm the previous literature. Of note, the improvement in dexterity was found to be related to a better prognosis while the crude strength was not. This could be related to our study group characteristics, which included patients with mild to moderate arm impairment. The strength itself is a measure of the force recovery that indeed was noted between the basal and the day 7 study, but, the recovery of fine movements and dexterity reflects the tuning of the survived motoneurons, which do correlate with functional outcome.

6.3 Objective 2.2. To identify features of the motor evoked potential related to good prognosis

As related above, the MEP presence itself in acute stroke relates strongly to a good functional outcome.

We decided to take a step further trying to identify which of the features of the MEP could better forecast a good outcome in our patients, and at what time slot. Previous studies already reported that patients with MEPs present on initial testing had consistently higher functional scores throughout the study and better recovery at 12 months, whereas those with absent MEPs had high probability of death and poor

functional outcome (74). Moreover a gradual recovery of MEP amplitude over the course of 3 months was related to a better outcome. However no data on the degree of improvement of the neurophysiological parameters has been provided so far. Our analysis showed that it is clear that no variations means no functional improvement. On the other side, in patients with a better prognosis a slight but significant improvement in MEP amplitude and a shortening of latency was observed, both in proximal and distal muscles.

When analyzed separately we found that even a slight increase of MEP amplitude (0.5mV) in our group, was significantly related to a better prognosis with a very high specificity and sensibility both at proximal and distal level. However no great relationship was found for MEP latencies. Our hypothesis is that in a lesioned hemisphere, even a little recovery of the motoneuron pool or the CST translates in a small improvement in the MEP that, in turn, transcribes into a better motor function and functional recovery.

6.4 Objective 2.3. To describe the features of sensory impairment in acute stroke patients

To the best of our knowledge this is the first study prospectively evaluating the sensory function in acute stroke patients by a standardized test. Previous studies employed qualitative measures, or monofilaments (104-109). We decided to use electrical current with specific characteristic already utilized in sensory perception studies due to the activation of both the spinothalamic and proprioceptive bundles and the outcome is an absolute number with a high interindividual reproducibility. A part from this, we investigated both sensory and pain perception because of the different cortical areas involved in both sensations.

Our data provide several information:

1. As expected, sensory and pain threshold are strongly higher in the hand corresponding to the lesioned hemisphere compared to the contralateral

2. Patients with a better outcome show a lower threshold for both sensory and pain sensation compared to patients with a worse outcome, both at basal and day 7 study
3. In patients with preserved MEP both sensory and pain threshold were significantly lower irrespective of the muscle studied or the time slot.
4. The improvement in both thresholds between the 2 studies was found to be associated to a better functional outcome.

Together, this is the neurophysiological reflection of the deep relationship between motor and sensory function in the human brain(104,105). Indeed, patients with less impairment could be the ones with smaller stroke in terms of volume of the affected area and in turn be the patients with less motor and sensory involvement. However, it is interesting to think that motor and sensory recoveries go entangled and as such, the functional prognosis might be predicted by a quick test performable with any EMG equipment.

6.5 Limitations

The main limitations of this study are listed as follows:

1. Only moderate to mild acute stroke patients were included. Doing so, we excluded both very severe or very mild patients that could have provided a better clinical picture of the acute stroke. However, from previous study, it is known that severe very large stroke patients usually do not present a valid MEP, as well as very mild stroke do not alter the neurophysiology in general, so the analysis might have been biased including those patients.
2. Due to the large number of variables explored in this study, it was not possible to analyze all the associations among them. For this reason, we limited our analysis to the significant associations between neurophysiological features in acute stroke and outcome.
3. We could not retrieve prospective information on the rehabilitation treatment implemented in our study group. Probably they were treated differently

according to the functional status (bedded, walker etc) that could have of course modified the outcome of our patients. But this could reflect the reality of clinical care, where patients are usually treated according to their capabilities, adapting the rehabilitation to the improvement of each patient if present.

6.6 Strengths

The main strengths of this study are:

1. Patients' selection: The patients included in our study were prospectively collected during the hyperacute phase and the study was conducted in the first 48-72h since stroke onset. Moreover, we included only patients with mild and moderate motor impairment avoiding recovered patients and too severe patients.
2. The comprehensive neurophysiological approach: we explored in both the acute phase and at day 7 several neurophysiological measures both motor (cortical excitability, MEP, cSP) and sensory, which was not explored previously in the same group of patients.
3. Motor evaluation: We used 2 scales to evaluate motor performance. The BMC which describes strength in separated muscular groups and the Fugl-Meyer scale, which explores the dexterity and the motor performance with several pre-defined movement. Doing so, we collected data on both the crude muscle force and on performance.

7. CONCLUSIONS

The conclusions emerged from our work are the followings:

1. Acute stroke provokes profound changes in the cortical function and pyramidal tract. Those changes are strictly related to functional outcome at 3-months
2. Apart from MEP presence itself, an amplitude increase of 0.5mV between basal and day 7 studies is related to a better functional outcome at 3months
3. The degree of sensory impairment is related to the functional outcome, and it is strictly related to motor function basally and with motor function evolution

8. FUTURE LINES OF INVESTIGATION

This study provides a comprehensive evaluation of acute stroke patients. However, we provide hints on which patients will have a better prognosis with conventional care.

Neuromodulation therapy both with repetitive TMS or direct current stimulation are growing incessantly since their introduction and although controversial results have been provided recently in stroke patients(112–115), the application of tailored treatments based on neurophysiological measures could be the future.

We provide the means to identify such patients that, although moderately impaired, will not improve with standard care. So, those patients might benefit from a more intensive rehabilitation care, or from the employment of new therapies, such as neuromodulation, or of course, the combination of the 2.

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10. APPENDIX

Appendix 1. Fugl Meyer Scale

FMA-UE PROTOCOL — Rehabilitation Medicine, University of Gothenburg

FUGL-MEYER ASSESSMENT
UPPER EXTREMITY (FMA-UE)
Assessment of sensorimotor function

ID:
Date:
Examiner:

Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, Steglind S: The post-stroke hemiplegic patient. A method for evaluation of physical performance. Scand J Rehabil Med 1975, 7:13-31.

A. UPPER EXTREMITY, sitting position					
I. Reflex activity				**none**	**can be elicited**
Flexors: biceps and finger flexors (at least one)				0	2
Extensors: triceps				0	2
			Subtotal I (max 4)		
II. Volitional movement within synergies, without gravitational help				**none**	**partial**	**full**
Flexor synergy: Hand from contralateral knee to ipsilateral ear. From extensor synergy (shoulder adduction/ internal rotation, elbow extension, forearm pronation) to flexor synergy (shoulder abduction/ external rotation, elbow flexion, forearm supination). **Extensor synergy:** Hand from ipsilateral ear to the contralateral knee	Shoulder	retraction		0	1	2
		elevation		0	1	2
		abduction (90°)		0	1	2
		external rotation		0	1	2
	Elbow	flexion		0	1	2
	Forearm	supination		0	1	2
	Shoulder	adduction/internal rotation		0	1	2
	Elbow	extension		0	1	2
	Forearm	pronation		0	1	2
			Subtotal II (max 18)			
III. Volitional movement mixing synergies, without compensation				**none**	**partial**	**full**
Hand to lumbar spine hand on lap	cannot perform or hand in front of ant-sup iliac spine			0		
	hand behind ant-sup iliac spine (without compensation)				1	
	hand to lumbar spine (without compensation)					2
Shoulder flexion 0°- 90° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion			0		
	abduction or elbow flexion during movement				1	
	flexion 90°, no shoulder abduction or elbow flexion					2
Pronation-supination elbow at 90° shoulder at 0°	no pronation/supination, starting position impossible			0		
	limited pronation/supination, maintains starting position				1	
	full pronation/supination, maintains starting position					2
			Subtotal III (max 6)			
IV. Volitional movement with little or no synergy				**none**	**partial**	**full**
Shoulder abduction 0 - 90° elbow at 0° forearm pronated	immediate supination or elbow flexion			0		
	supination or elbow flexion during movement				1	
	abduction 90°, maintains extension and pronation					2
Shoulder flexion 90° - 180° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion			0		
	abduction or elbow flexion during movement				1	
	flexion 180°, no shoulder abduction or elbow flexion					2
Pronation/supination elbow at 0° shoulder at 30°- 90° flexion	no pronation/supination, starting position impossible			0		
	limited pronation/supination, maintains start position				1	
	full pronation/supination, maintains starting position					2
			Subtotal IV (max 6)			
V. Normal reflex activity assessed only if full score of 6 points is achieved in part IV; compare with the unaffected side				**0 (IV), hyper**	**lively**	**normal**
biceps, triceps, finger flexors	2 of 3 reflexes markedly hyperactive or 0 points in part IV			0		
	1 reflex markedly hyperactive or at least 2 reflexes lively				1	
	maximum of 1 reflex lively, none hyperactive					2
			Subtotal V (max 2)			
			Total A (max 36)			

Approved by Fugl-Meyer AR 2010 — 1 — Updated 2015-03-11

B. WRIST support may be provided at the elbow to take or hold the starting position, no support at wrist, check the passive range of motion prior testing		none	partial	full
Stability at 15° dorsiflexion elbow at 90°, forearm pronated shoulder at 0°	less than 15° active dorsiflexion dorsiflexion 15°, no resistance tolerated maintains dorsiflexion against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 90°, forearm pronated shoulder at 0°, slight finger flexion	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Stability at 15° dorsiflexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	less than 15° active dorsiflexion dorsiflexion 15°, no resistance tolerated maintains dorsiflexion against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Circumduction elbow at 90°, forearm pronated shoulder at 0°	cannot perform volitionally jerky movement or incomplete complete and smooth circumduction	0	1	2
	Total B (max 10)			

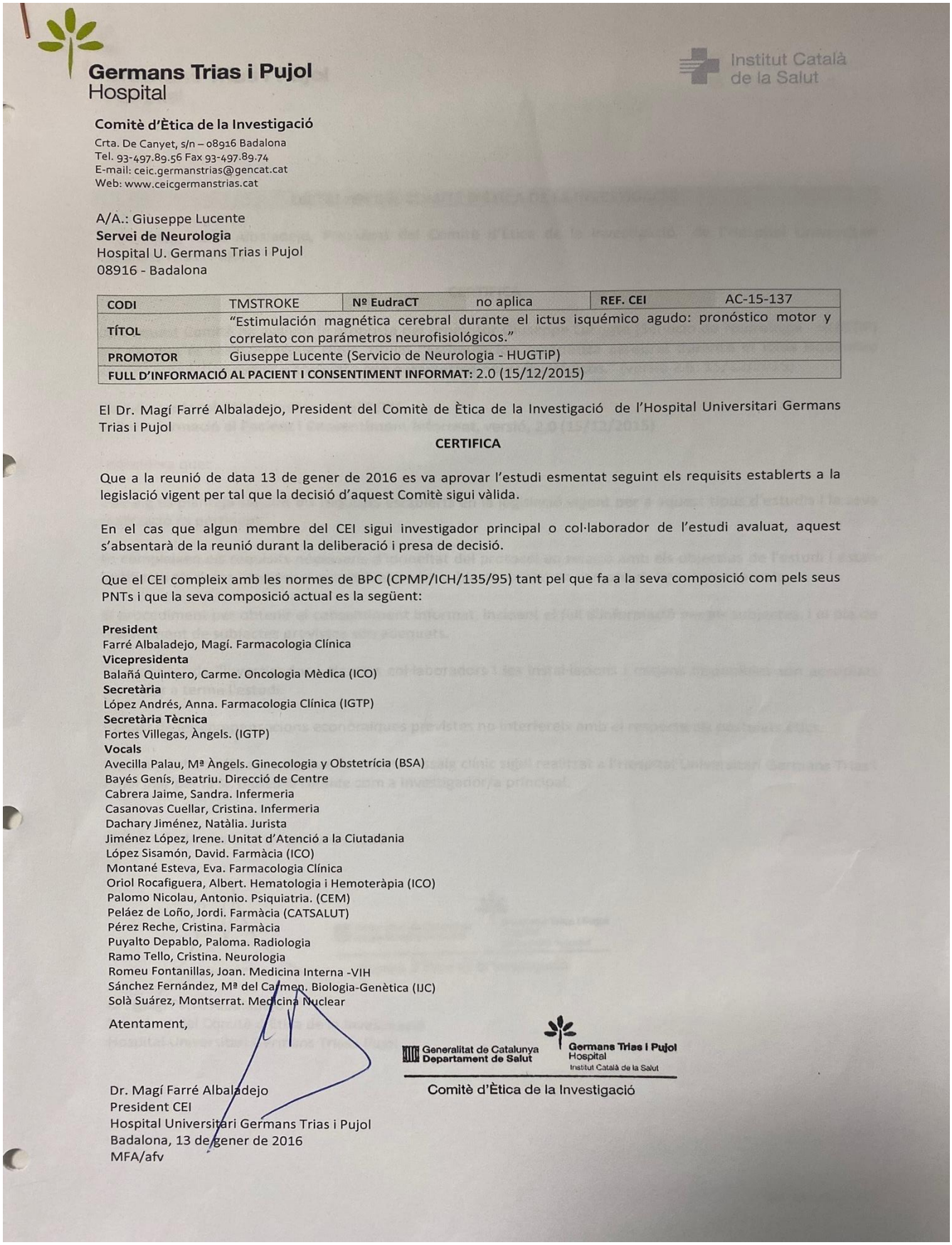
C. HAND support may be provided at the elbow to keep 90° flexion, no support at the wrist, compare with unaffected hand, the objects are interposed, active grasp		none	partial	full
Mass flexion from full active or passive extension		0	1	2
Mass extension from full active or passive flexion		0	1	2
GRASP				
a. Hook grasp flexion in PIP and DIP (digits II-V), extension in MCP II-V	cannot be performed can hold position but weak maintains position against resistance	0	1	2
b. Thumb adduction 1-st CMC, MCP, IP at 0°, scrap of paper between thumb and 2-nd MCP joint	cannot be performed can hold paper but not against tug can hold paper against a tug	0	1	2
c. Pincer grasp, opposition pulpa of the thumb against the pulpa of 2-nd finger, pencil, tug upward	cannot be performed can hold pencil but not against tug can hold pencil against a tug	0	1	2
d. Cylinder grasp cylinder shaped object (small can) tug upward, opposition of thumb and fingers	cannot be performed can hold cylinder but not against tug can hold cylinder against a tug	0	1	2
e. Spherical grasp fingers in abduction/flexion, thumb opposed, tennis ball, tug away	cannot be performed can hold ball but not against tug can hold ball against a tug	0	1	2
	Total C (max 14)			

D. COORDINATION/SPEED, sitting, after one trial with both arms, eyes closed, tip of the index finger from knee to nose, 5 times as fast as possible		marked	slight	none
Tremor	at least 1 completed movement	0	1	2
Dysmetria at least 1 completed movement	pronounced or unsystematic slight and systematic no dysmetria	0	1	2
		≥ 6s	**2 - 5s**	**< 2s**
Time start and end with the hand on the knee	at least 6 seconds slower than unaffected side 2-5 seconds slower than unaffected side less than 2 seconds difference	0	1	2
	Total D (max 6)			

Appendix 2. British Medical Council Muscular Scale

Grade 0	No movement is observed
Grade 1	Only a trace or flicker of movement is seen or felt in the muscle, or fasciculation is observed
Grade 2	Movement is possible only if the resistance of gravity is removed
Grade 3	Movement against gravity is possible but not against resistance of the examiner
Grade 4	Muscle strength is reduced but muscle contraction can move joint against gravity and resistance
Grade 5	Muscle contracts normally against full resistance

Appendix 3. Ethic Committee Approval



Germans Trias i Pujol
Hospital

Institut Català de la Salut

Comitè d'Ètica de la Investigació

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A/A.: Giuseppe Lucente
Servei de Neurologia
Hospital U. Germans Trias i Pujol
08916 - Badalona

CODI	TMSTROKE	**Nº EudraCT**	no aplica	**REF. CEI**	AC-15-137
TÍTOL	"Estimulación magnética cerebral durante el ictus isquémico agudo: pronóstico motor y correlato con parámetros neurofisiológicos."				
PROMOTOR	Giuseppe Lucente (Servicio de Neurologia - HUGTiP)				
FULL D'INFORMACIÓ AL PACIENT I CONSENTIMENT INFORMAT: 2.0 (15/12/2015)					

El Dr. Magí Farré Albaladejo, President del Comitè de Ètica de la Investigació de l'Hospital Universitari Germans Trias i Pujol

CERTIFICA

Que a la reunió de data 13 de gener de 2016 es va aprovar l'estudi esmentat seguint els requisits establerts a la legislació vigent per tal que la decisió d'aquest Comitè sigui vàlida.

En el cas que algun membre del CEI sigui investigador principal o col·laborador de l'estudi avaluat, aquest s'absentarà de la reunió durant la deliberació i presa de decisió.

Que el CEI compleix amb les normes de BPC (CPMP/ICH/135/95) tant pel que fa a la seva composició com pels seus PNTs i que la seva composició actual es la següent:

President
Farré Albaladejo, Magí. Farmacologia Clínica
Vicepresidenta
Balañá Quintero, Carme. Oncologia Mèdica (ICO)
Secretària
López Andrés, Anna. Farmacologia Clínica (IGTP)
Secretària Tècnica
Fortes Villegas, Àngels. (IGTP)
Vocals
Avecilla Palau, Mª Àngels. Ginecologia y Obstetrícia (BSA)
Bayés Genís, Beatriu. Direcció de Centre
Cabrera Jaime, Sandra. Infermeria
Casanovas Cuellar, Cristina. Infermeria
Dachary Jiménez, Natàlia. Jurista
Jiménez López, Irene. Unitat d'Atenció a la Ciutadania
López Sisamón, David. Farmàcia (ICO)
Montané Esteva, Eva. Farmacologia Clínica
Oriol Rocafiguera, Albert. Hematologia i Hemoteràpia (ICO)
Palomo Nicolau, Antonio. Psiquiatria. (CEM)
Peláez de Loño, Jordi. Farmàcia (CATSALUT)
Pérez Reche, Cristina. Farmàcia
Puyalto Depablo, Paloma. Radiologia
Ramo Tello, Cristina. Neurologia
Romeu Fontanillas, Joan. Medicina Interna -VIH
Sánchez Fernández, Mª del Carmen. Biologia-Genètica (IJC)
Solà Suárez, Montserrat. Medicina Nuclear

Atentament,

Generalitat de Catalunya
Departament de Salut

Germans Trias i Pujol
Hospital
Institut Català de la Salut

Comitè d'Ètica de la Investigació

Dr. Magí Farré Albaladejo
President CEI
Hospital Universitari Germans Trias i Pujol
Badalona, 13 de gener de 2016
MFA/afv

Germans Trias i Pujol
Hospital

Institut Català de la Salut

DICTAMEN DEL COMITÈ D'ÈTICA DE LA INVESTIGACIÓ

El Dr. Magí Farré Albaladejo, President del Comitè d'Ètica de la Investigació de l'Hospital Universitari Germans Trias i Pujol

CERTIFICA

Que aquest Comitè ha avaluat la proposta del promotor Giuseppe Lucente (Servicio de Neurologia - HUGTiP) per tal que es realitzi l'assaig clínic, titulat "**Estimulación magnética cerebral durante el ictus isquémico agudo: pronóstico motor y correlato con parámetros neurofisiológicos.**" (versió 2.0: 15/12/2015)

Codi de protocol del promotor: TMSTROKE,
Full d'Informació al Pacient i Consentiment Informat, versió, 2.0 (15/12/2015)

i considera que:

L'assaig es planteja seguint els requisits establerts en la legislació vigent per a aquest tipus d'estudis i la seva realització és pertinent.

Es compleixen els requisits necessaris d'idoneïtat del protocol en relació amb els objectius de l'estudi i estan justificats els riscos i molèsties previsibles per al subjecte, tenint en compte els beneficis esperats.

El procediment per obtenir el consentiment informat, incloent el full d'informació per als subjectes, i el pla de reclutament de subjectes previstos són adequats.

La capacitat de l'investigador i els seus col·laboradors i les instal·lacions i mitjans disponibles són apropiats per a dur a terme l'estudi.

L'abast de les compensacions econòmiques previstes no interfereix amb el respecte als postulats ètics.

I que aquest Comitè accepta que l'esmentat assaig clínic sigui realitzat a l'Hospital Universitari Germans Trias i Pujol pel/ per la Sr. Giuseppe Lucente com a investigador/a principal.

Signat a Badalona a 13 de gener de 2016

Generalitat de Catalunya
Departament de Salut

Germans Trias i Pujol
Hospital
Institut Català de la Salut

Comitè d'Ètica de la Investigació

Dr. Magí Farré Albaladejo
President del Comitè d'Ètica de la Investigació
Hospital Universitari Germans Trias i Pujol

Direcció centre
Crta. De Canyet, s/n
08916 Badalona

CONFORMIDAD DE LA DIRECCIÓN DEL CENTRO

La Dra. Beatriu Bayés Genís, Directora del Hospital Universitario Germans Trias i Pujol, y vista la autorización del Comité de Ética de la Investigación.

C E R T I F I C A

Que conoce la propuesta realizada por **Giuseppe Lucente (Servicio de Neurologia - HUGTiP)**, para que sea realizado en este Centro el estudio titulado:

"Estimulación magnética cerebral durante el ictus isquémico agudo: pronóstico motor y correlato con parámetros neurofisiológicos." versión 2.0 (15/12/2015)

Código de protocolo **TMSTROKE**
nº EudraCT: **no aplica**

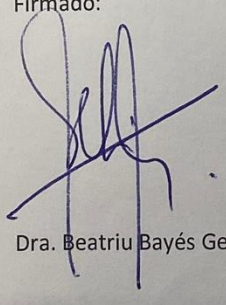
Que será realizado por el **Dr. Giuseppe Lucente** del Servicio de Neurología como investigador principal.

Que está de acuerdo con su viabilidad desde el punto de vista económico.

Que acepta la realización de dicho estudio en este Centro.

Lo que firma en Badalona a 18 de enero de 2016

Firmado:



Dra. Beatriu Bayés Genís

REF. CEI: **AC-15-137**

A