



Parameters Characterization and Cognitive-Behavioral Effects of Transcranial Pulsed Current Stimulation

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STATEMENT

I declare that the work presented in this thesis is my own. No portion of this work was submitted in support of an application for another degree of this or any other university, or institute of learning.

Jorge León Morales Quezada MD, MSC.

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ABBREVIATIONS and ACRONYMS

AC	Alternate current
AMPA	Amino-hydroxy-methylisoxazol-propanoic acid
AST	Attention switching task
BDNF	Brain derived neurotrophic factor
BOLD	Blood-oxygen-level-dependent
CES	Cranial electrical stimulation
CNS	Central Nervous system
DC	Direct current
DLPFC	Dorsolateral prefrontal cortex
ECT	Electroconvulsive therapy
EEG	Electroencephalography
EPSP	Excitatory postsynaptic potential
FDA	Food and drug administration
FFT	Fast Fourier transformation
FES	Functional electrical stimulation
fMRI	Functional magnetic resonance

GABA Gamma-aminobutyric acid

HRV Heart rate variability

LTD Long term depression

LTP Long term potentiation

MMSE Mini mental state examination

NIBS Noninvasive brain stimulation

NMDA N-Methyl-D-aspartic acid

PALT Paired associative learning task

PET Positron emitting tomography

PFC Prefrontal cortex

PNS Peripheral nervous system

PMA Premotor associative

qEEG Quantitative electroencephalography

tACS Transcranial alternating current stimulation

tDCS Transcranial direct current stimulation

tPCS Transcranial pulsed current stimulation

TMS Transcranial magnetic stimulation

ABSTRACT

Neuromodulation is being recognized as “technology impacting on the neural interface” And noninvasive brain stimulation (NIBS) is becoming an interesting alternative for this interface. Transcranial pulsed current stimulation (tPCS) is emerging as an option in the field of neuromodulation as a technique that employs weak, pulsed current at different frequency ranges, inducing electrical fields that reach cortical and subcortical structures; however, little is known about its properties and mechanistic effects on electrical brain activity and how it can modulate its oscillatory patterns. Moreover, there is not clear understanding in how tPCS can affect cognition and behavior or its neurophysiological correlates as indexed by autonomic responses.

This research looked at the mechanisms behind tPCS in four randomized clinical trials; the main aim of each experiment was to evaluate the effects of tPCS in quantitative electroencephalography (qEEG) and cognitive-behavioral testing by exploring different parameters of stimulation. Based in the findings obtained per experiment, tPCS can be defined as a safe and tolerable technique that modulates the power spectrum of qEEG signals by means of applied randomized frequencies in a pre-defined range, tPCS also facilitates connectivity in the area of influence by the electrical field and this has an impact on optimization of performance by decreasing reaction times (RT) in attention switching task and by facilitating wide-ranging network processing as in the case of arithmetic functioning.

This work also delivered an insight about the potential that tPCS has for future clinical applications.

CONTENTS

1. INTRODUCTION	11
1.1 Background.....	11
1.2 Definitions.....	16
1.3 History	20
1.4 Research Problem	26
1.5 Objective and Aims	27
1.5 Thesis structure	29
2. LITERATURE REVIEW.....	30
2.1 Introduction	30
2.2 Principles of Electrical Stimulation	31
2.3 Neurophysiology	34
2.3 Neuroplasticity.....	38
2.4 Physiological basis of Electroencephalogram (EEG).....	44
2.5 Quantitative Analysis of the EEG (QEEG).....	49
2.6 Relevant Functional Neuroanatomy.....	53
2.7 Transcranial Direct Current Stimulation and Transcranial Magnetic Stimulation as a model of noninvasive brain stimulation	60
3. METHODOLOGY.....	67
3.1 Experiment 1 (defining stimulation frequency)	69
3.2 Experiment 2 (defining stimulation intensity).....	81
3.3 Experiment 3 (cognitive behavioral and autonomic responses)	86
3.4 Experiment 4 (cognitive behavioral and autonomic responses)	95
4. RESULTS.....	102
4.1 Experiment 1 (definition of frequency).....	102
4.2 Experiment 2 (defining stimulation intensity).....	107
4.3 Experiment 3 (cognitive behavioral and autonomic responses)	111
4.4 Experiment 4 (cognitive behavioral and autonomic responses)	116
5. DISCUSSION.....	121
5.1 Experiment 1	122
5.2 Experiment 2	125
5.3 Experiment 3	128

5.4 Experiment 4	134
6. LIMITATIONS	140
7. CONCLUSIONS.....	142
References.....	146

LIST OF FIGURES

Figure 1. 1. tPCS current modeling	12
Figure 1. 2. Sinusoidal wavelength and quadratic biphasic pulse	13
Figure 1. 3. Original traces from evoked sensory motor potentials	23
Figure 1. 4. Effects in the amplitude of motor evoked potentials	25
Figure 2. 1. Schematic representation of current flow in a direct current montage	34
Figure 2. 2. Ionic equilibrium for the generation of an action potential	35
Figure 2. 3. Intra and extracellular membrane polarity changes.....	36
Figure 2. 4. Neurodevelopmental model of plasticity	42
Figure 2. 5. Biological mechanism affecting neoplastic processes.....	43
Figure 2. 6. International 10/20 system for EEG recording.....	45
Figure 2. 7. Frequency bands	47
Figure 2. 8. Raw EEG signal and spectral analysis	52
Figure 2. 9. Computational current modeling of tPCS	53
Figure 2. 10. Prefrontal cortex	56
Figure 2. 11. BOLD activation maps	64
Figure 3. 1. Study flow	69
Figure 3. 2. tPCS apparatus.....	73
Figure 3. 3. Pulse frequency registration.....	73
Figure 3. 4. Study design	88
Figure 3. 5. Balloon analog risk task.....	91
Figure 3. 6. Arithmetic task	92
Figure 3. 7. PALT and AST design.....	97
Figure 3. 8. Cognitive task	100
Figure 5 1. Effect of tPCS theta and low Alpha coherence	106
Figure 5 2. Interhemispheric coherence of theta and low alpha	109
Figure 5 3. Arithmetic task	112
Figure 5. 4. BART task	113
Figure 5. 5. Heart variability	115
Figure 5. 6. Attention switching task	117
Figure 5. 7. Paired associative learning task	118

Figure 5. 8. LF/HF	119
Figure 5. 9. EDR measurements	120

LIST OF TABLES

Table 2. 1. Typical positron emission tomography/functional magnetic resonance imaging activations during memory tests.....	60
Table 3. 1. Experiments and assessments schematic representation.....	68
Table 3. 2. Demographic data at baseline. Mean (SD)	70
Table 3. 3. Demographic data at baseline. Mean (SD)	82
Table 3. 4. Demographic data at baseline. Mean (SD).....	96

PEER-REVIWED PUBLICATIONS

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PRESENTATIONS

1. VI International Neuromodulation Symposium. Lecture “Combined Protocols in Neurological Rehabilitation” In this lecture tPCS was presented as an option for noninvasive brain stimulation. Sao Paulo, Brazil. August 2014.
2. VII International Neuromodulation Symposium. Lecture “Neurophysiological Mechanisms of Transcranial Pulsed Current Stimulation” Workshop “Applications of Transcranial Pulsed Current Stimulation for Cognitive Performance” Sao Paulo Brazil, 2015.
3. 19th Annual Meeting North America Neuromodulation Society. Lecture “QEEG Guided Noninvasive Brain Stimulation” In this lecture tPCS was included as therapeutic technique. December 2015, Las Vegas, Nevada. USA.

1. INTRODUCTION

In this chapter a summary account of the background and rationale that lead to the development of this investigation, it will also present the main aim and objectives and the significance of this research has for the development of the field. Finally, an outline of the entire thesis is offered.

1.1 Background

Neuroscience as a basic and applied discipline is uncovering and revolutionizing the way we understand and conceive the function of the human brain, from a rudimentary technique yet perfectly stained neurons created from Santiago Ramon y Cajal to the most sophisticated system for brain imaging currently available in most specialized hospitals around the world. Still, there is a vast amount of ground to cover when exploring the interactions between neural systems and external physical forces. It is the role of neuromodulation, an applied division of the neurosciences that is leading the progress in this domain by applying invasive and noninvasive methods for brain and nerve stimulation.

The contemporary pioneering work of Bindman helped to understand how short electrical currents affects the physiology, initially in muscle (Bindman, Lippold, & Redfearn, 1962) to later explore those effects on the rat's brain (L. J. Bindman, O. Lippold, & J. Redfearn, 1964), since then, several techniques and devices to provide electrical stimulation have been used to modulate brain activity, the first approved technique to be used in humans for the treatment of depression and bipolar disorder was the Electroconvulsive Therapy (ECT), unfortunately the controversy that surrounds this technique has affected negatively the overall development of the noninvasive brain stimulation (NIBS) methods (Lauber, Nordt, Falcató, & Rossler, 2005).

During the last 20 years we have witnessed the rediscovering and development of noninvasive brain stimulation (NIBS); techniques such as transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) have flourished thanks to the advancements in functional imaging, elegant neurophysiological assessments, computer generated modeling, and most importantly, due to the groundwork of well-designed research methodology. Another technique of non-invasive brain stimulation is transcranial pulsed current stimulation (tPCS) - also known as transcranial alternating current stimulation (tACS), or cranial electrotherapy stimulation (CES) that has FDA clearance for its application in the treatment of depression, insomnia and anxiety – it has shown to be a useful technique to modulate cortical and subcortical neural circuits as recently shown by our group of collaborators from City University of New York (Datta., et al, 2013) where current behavior was modeled using computer simulation methods (figure 1.1). Because its safe profile, ease of use (requires only placement of ear clips) and the proposed neural effects, tPCS is a good candidate for further development of the technique that may provide potential as therapeutic tool in neurological rehabilitation for the treatment of chronic pain and cognitive dysfunction associated with brain lesion.

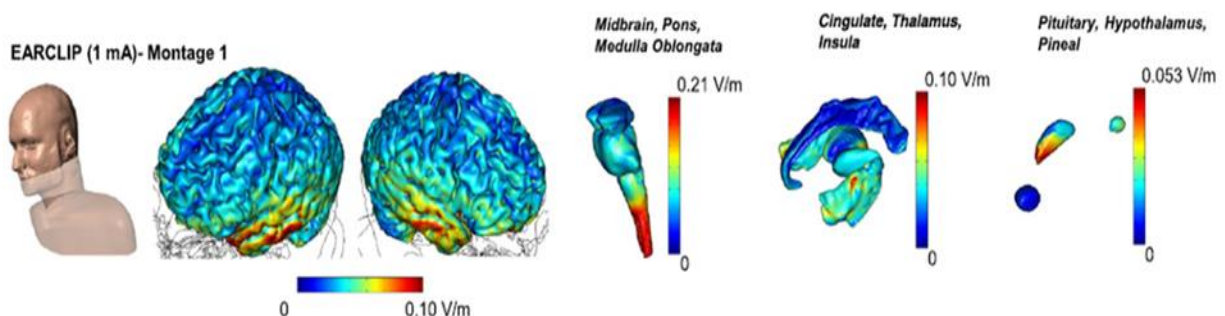


Figure 1. 1. tPCS current modeling using bilateral earclip electrode montage, showing current distribution in temporo-prefrontal cortex, and subcortical structures. From Datta., et all. 2013.

tPCS devices use dose parameters typically between the range of 50 μA to 5 mA current intensity, and around wide array of frequencies on Hz, it is typically applied over a multiple sessions of 20 to 30 minutes, and is being applied using surface electrodes on the infra- or supra-auricular structures (Zaghi, Acar, Hultgren, Boggio, & Fregni, 2010). Although tPCS has been used for several decades (Edelmuth, Nitsche, Battistella, & Fregni, 2010) it has been reported to be effective for the treatment of insomnia, depression and anxiety in several clinical studies, the mechanisms of action remain unknown.

In summary, tPCS involves the application of pulsed, low-amplitude electrical current to the head using electrodes clipped to the earlobes (most conventional montage). The current comes from a battery source that has a high frequency cycling design. Thus, using the nomenclature adopted in the field of NIBS, tPCS is a specific type of transcranial alternating current with a biphasic temporal pulses (figure 1.2). The user can increase the intensity from 10 up to 500 millionths of an ampere, but the frequency is usually set at 0.5 Hz. Since tPCS generates an alternating bidirectional current, it does not matter where the anode or cathode are positioned following a bilateral pattern (earlobes). The standard “recommended” session lasts 20 minutes per day, but a session can go as long as 60 minutes if needed.

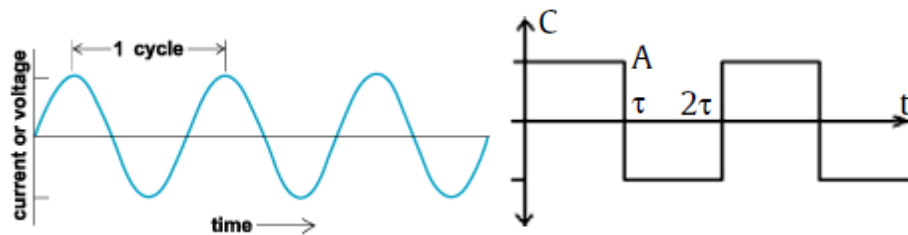


Figure 1. 2. Differences in a sinusoidal wavelength and a quadratic biphasic pulse. Adapted from Datta, et all. 2013

Few studies have shown the effects of tPCS on brain activity, and it is still unclear its neural mechanisms of action. It is postulated that the stimulation of brain tissue causes increased amounts of neurotransmitters to be released, specifically serotonin, beta endorphin, and norepinephrine (Y. F. Shealy, 1989). These neurotransmitters, in turn, permit a return to normal biochemical homeostasis of the limbic system of the brain that may have been imbalanced by a stress-related condition. However, most of the proposed mechanisms of action still mere assumptions based in surrogate animal models from other NIBS techniques, especially from traditional tACS where the alternating nature of the current is the only common denominator if compared with tPCS.

In this study, the researcher aims to address the gaps in the investigation of this technique – including characterization of optimal parameters to induce cognitive modulation, and evaluation of its mechanisms of action. These will be indexed by changes in qEEG, and cognitive testing measures.

In determining how tPCS can best be used as a therapeutic tool, it is important to quantify and correlate electroencephalographic (EEG) changes with the administration of various tPCS waveform shapes, frequencies, and intensities. Although there has been research to investigate quantitative EEG changes before and after tPCS application (Itil, Saletu, & Davis, 1972), minimal literature exists on quantitative EEG changes *during* the application of tPCS (Ferdjallah, Bostick, & Barr, 1996). This is due mostly to the difficulty of eliminating the stimulating signal from the EEG. Schroeder & Barr showed qEEG changes in the power of alpha and beta bands after tPCS at 0.5 and 100 Hz for 20 minutes. Their results where significant for presenting down-regulation of the alpha band with both frequencies and the 100Hz condition produced down-regulation shift in the alpha and beta median frequency and band power fraction respectively

(Schroeder & Barr, 2001). More recently, Datta et al, using a sophisticated computer based high-resolution current modeling, evaluated different electrode montages, their results confirmed that significant amount of current pass the skull and reach cortical and subcortical structures (Abhishek Datta, Jacek P. Dmochowski, Berkan Guleyupoglu, Marom Bikson, & Felipe Fregni, 2013).

The activity of neuronal networks in the mammalian forebrain demonstrates several oscillatory bands covering frequencies from approximately 0.05 Hz to 500 Hz. The mean frequencies of the experimentally observed oscillator categories form a linear progression on a natural logarithmic scale with a constant ratio between neighboring frequencies, leading to the separation of frequency bands. Neighboring frequency bands within the same neuronal network are typically associated with different brain states and compete with each other (Engel, 2001. Klimesch, 1999). On the other hand, several rhythms can temporally coexist in the same or different structures and interact with each other (Grenier, Timofeev, & Steriade, 2001). The power density of EEG or local field potential is inversely proportional to frequency (f) in the mammalian cortex. This $1/f$ power relationship implies that perturbations occurring at slow frequencies can cause a cascade of energy dissipation at higher frequencies (Bak, 1987) and those widespread slow oscillations modulate faster local events. These properties of neuronal oscillators are the result of the physical architecture of neuronal networks and the limited speed of neuronal communication due to axon conduction and synaptic delays (Nunez, 1995). Because most neuronal connections are local (Braitenberg, 1998), the period of oscillation is constrained by the size of the neuronal pool engaged in a given cycle. tCPS may offer the possibility to modulate the network oscillatory properties by introducing transcranial carrier frequencies that

resemble those observed by cognitive processing, thus network connectivity and coherence may be enhanced by exogenous stimulation and cognitive training.

Some preliminary evidence suggests that AC brain stimulation is able to alter and improve cognitive skills (Polanía, 2012). It seems plausible that the use of tPCS as a neuromodulation technique can induce dynamic changes in cortical areas including those responsible for cognitive function in both healthy subjects and those with neurological or psychiatric conditions, leading to changes in learning processes as a result of memory modulation or in decision-making responses, attention and performance by circuitry enhancement. The idea of using this stimulation technique as a potential therapeutic method for neurocognitive deficits with alterations in the physiology of the network connectivity opens a new window to future research.

We are in a period where the establishment of optimal stimulation parameters is necessary for reliable cortical-subcortical neuromodulation, also by understanding the dynamics of the proposed parameters; we will be able to correlate its mechanisms of function for possible application of this technique to cognitive modulation.

1.2 Definitions

To understand the role that noninvasive brain stimulation NIBS has in cognitive neurosciences, first, we need to define the terminology that from now on is going to be used in these writings.

At first glance it might give the impression that some terms are interchangeable used among different scientific disciplines, however, what can have a specific meaning in psychology

not necessary will be applied to what is understood in biophysics or neurology. Hence, terminology will follow the convention used in most published work in the field of neuromodulation. Neuromodulation as an emergent field in neurosciences is transforming how we conceptualize the function and processes that happen in the central (CNS) and peripheral (PNS) nervous system, it is also opening new venues for the treatment of some neuropsychiatric conditions or as a booster of cognitive functioning in healthy population.

Neuromodulation is among the fastest-growing areas of medicine, involving many diverse specialties and impacting hundreds of thousands of patients with numerous disorders worldwide. In the past decade, neuromodulation has witnessed significant advances with regard to the science, mechanisms, clinical applications, and technology development (Krames, Peckham, & Rezai, 2009). But is not only in medicine where neuromodulation is changing the way some conditions are being treated, it is also in neuropsychology where we can observe explicit changes in behavioral traits as this technology alters the function of specific areas in the brain, or in cognitive neurosciences where by using different neuromodulatory techniques the operator can either facilitate or inhibit learning processes or promote consolidation of previously learned knowledge in healthy or neurologically ill individuals.

The International Neuromodulation Society (Sakas, Panourias, Simpson, & Krames, 2007) defines neuromodulation as a field of science, medicine, and bioengineering that encompasses implantable and non-implantable technologies, electrical or chemical, for the purpose of improving quality of life and functioning of humans. It is at this point where biomedical sciences get close involved with exact physics and mathematics, especially for the application of electric, ultrasonic, light, heat or cold as physical energies to interact with biological tissue. For the purpose of this work, neuromodulation will refer to any induced action promoting a measurable

change in excitable neurological tissue (at central or peripheral level), specifically those changes induced by electrical currents. Jan Holsheimer (Holsheimer, 2003) suggests that for a therapy to be considered neuromodulation, the therapy must consist of the following:

1. The therapy must be dynamic, ongoing (continuous or intermittent) intervention, and not a short and non-recurring procedure.
2. The activity of specific neural networks is affected by the ongoing electrical stimulation or by ongoing neuropharmacological stimulation.
3. The clinical effect is continuously controllable by varying one or more stimulation parameters to satisfy a patient's need.

Based in the principles described above, one can realize that the most important characteristics for a neuromodulatory treatment are the interactions with naturally occurring – pathological and non-pathological– neural events, and the need to have full control over the stimulation parameters. These come as *personalized* care takes a gradual significance in the disciplines of neurology, psychiatry, and psychology.

By defining other terms associated with the use and application of electrical currents, we can understand that neuroaugmentation is the use of electrical stimulation to supplement or enhance the activity of the nervous system. Neurostimulation is the process or technology that applies electrical currents, in varying parameters, by means of implanted or noninvasive electrodes to achieve functional activation or inhibition of specific neuronal groups, cortical areas, pathways, or networks (Online Medical Dictionary; www.nlm.nih.gov/medlineplus/mplusdictionary.html).

Cognitive enhancement by means of neurostimulation refers to the effect of electrical currents have on boosting, promoting and/or potentiating specific cognitive functions contingent to the area being stimulated. Specifically, noninvasive brain stimulation has earned too much attention as there is an increased interest in the neurosciences to understand how cognitive enhancement are supported within networks and at what cost these improvements represents in the overall cerebral economy.

Noninvasive brain stimulation applies to all forms of externally applied energy in any of the above mentioned forms. The stimulation is usually done by attaching electrodes on the head as in the case of electrical currents, coils for the delivery of magnetic pulses, probes for ultrasound diffusion, or specialized light sources for its transmission throughout the scalp. NIBS has experienced a dramatic acceleration in the last 20 or so years and its development has been expedited thanks to the accessibility in technology and reliable methods for the assessment of the stimulation effects has on the brain, from digital electroencephalography (EEG) to functional magnetic resonance imaging (fMRI). Nowadays, it is easier to observe and measure those changes even during the stimulation period, and these “online” observations helped the consolidation of NIBS as a research technique in neurosciences.

It can be stated without much hesitation that the human brain is among the most complex organs in the mammal kingdom, the advanced specialization of its components and how communication is transmitted throughout networks, circuits, nuclei, and tracts is the vivid expression of an efficient evolution in a continuum of self-organization. Current advancements in technology has facilitated our understanding of how the brain works, moreover we have reached a privileged status where scientist can directly or indirectly manipulate or *modulate* the function of the CNS and PNS looking for unknown physiological responses or for therapeutic

purposes. This doctoral thesis will present the research done in developing a specific noninvasive technique for neuromodulation, this work will serve as a reference to understand the mechanisms behind this technique and will also provide an insight of its applications in cognitive neurosciences.

1.3 *History*

The field of NIBS has brought too much attention in the last 10 years, despite the fact that, contemporary research of electrical stimulation and brain modulation resurrected in the beginning of the 1950s (Kimel & Kavalier, 1950), nevertheless, the application of electrical currents to the brain dates further back to ancient times, as reported by Scribonius Largus in the 15 AD, Scribonius was a roman doctor from the court of Emperor Tiberius (Stillings, 1975) and he served as battle field physician. Scribonius approach for the treatment of chronic pain, migraine, depression and epilepsy consisted in the use of an electrical ray directly placed over the patients' head, as this electrical fish discharged its current the maladies seemed to improve its symptomatology. Without knowing it Scribonius was able to implement a rudimentary form of neuromodulation through an external source of electricity. Unfortunately for the history of NIBS, Scribonius altogether with greek and roman physicians thought the electrical ray healing attributes came as "special powers" originated in the animal and not by the interaction between electrical currents and excitable neural tissue, yet, the utilization of this particular treatment for such neurological diseases indicates a link between those "special powers" and the underlying mechanisms in the treated conditions, that these ancient clinicians appeared to be able to understand.

By the early eighteenth century the leading scientists still did not know what substance was flowing through nerves (Finger, 2000). The much celebrated character of Benjamin Franklin was among the first to observe muscle contraction as a response to an electrical shock (Isaacson, 2003) which antedated the scientific work of the Italian physician Luigi Galvani who in 1780 published his research done in electrical stimulation on frog muscles, *De viribus electricitatis in motu musculari. Commentarius. Pars prima*. Bolonien Scientiarium Art Inst Adad 1791 (Preul, 1997), still Galvani's work has the merit to bring the concept of natural electricity generated by an organic entity, his research demonstrated how an exposed nerve will successfully conduct electrical energy from a rudimentary external battery to a rather complex anatomical structure. After Galvani's seminal work in neurophysiology and electricity (hence the name of Galvanic current or Galvanism), Giovanni Aldini, Galvani's nephew and a physicist studying the effects of Galvanism in human anatomy (Aldini & Fournier, 1804), started a series of macabre experiments in corpses, while applying electrical currents to decapitated heads promoting muscle contraction of the facial muscles, obtaining jaw movements, grimaces, and even eye openings (Higgins & George, 2008). Much of Aldini's experiments favored a ghoulish development of popular folklore that influenced the work of Mary Shelley's most influent publication, that still relating the use of electricity and medical applications up to these days. Besides the fiction brought by Shelley's novel, Gustav Fritsch and Eduard Hitzig in late 1860s successfully mapped the motor cortex of dogs by using electrical stimulation (Fitsch & Hitzig, 1870).

For many Duchenne de Bologne is the "real" father of modern neurology as a medical discipline, but for the field of neuromodulation Duchenne de Boulogne is also the first clinician, who described the controlled use of galvanic stimulation, Duchenne was also the first author to introduce principles of electrophysiology and electrotherapy for clinical purposes in his book

called “*De l’électrisation localisée et de son application à la physiologie, à la pathologie et à la thérapeutique*”(Duchenne, 1855), Duchenne was recognized for pioneering many aspects of neurological diagnostics, he described many forms of paralysis and introduced the concept of biopsy for the study of pathology in neural tissue. In 1875, it was reported that Duchenne applied electrical stimulation to the lower extremities of a paraplegic patient, by doing this Duchenne aimed to modulate in a retrograde fashion the functioning of the higher order neuron while improving locally the strength of the patient’s muscles, this gave rise of what we know now as functional electrical stimulation (FES), after Duchenne’s work many researchers started to experiment with electricity as therapeutic tool, it also came the era of invasive brain stimulation where Dr. Roberts Bartholow (1874), Sir Victor Horsley (1886), and Harvey Cushing (1909) among others did a groundbreaking work in mapping the human cortex in alive patients, their work cemented the basis of a more sophisticated understanding of neuromodulation.

The twentieth century can be called the “electrical century” as this energy started to be used in industry, cities, in consumers’ products and in people’s home. It was the time that electricity represented the most innovative energy the future offered to the human beings, and medicine could not escape either to this fascination, “electrical doctors” applied electricity to treat pain locally, although most of them did not know how exactly the electrical current was able to control pain, this early “neuromodulators” understood that a physical force can be used to control pathological process, the early devices available for treatment represented a primitive form of what we know this days as TENS units (transcutaneous electrical nerve stimulation) one of them called the Electreat was sold for \$1.00 and it was battery powered, the indications for its use included but not restricted to promote “well-being”, pain control, increased blood flow, and provide muscle relaxation, although none of these statements were proven in controlled trials the

device gained popularity and even acceptance among the medical community, the Electreat was perhaps the first TENS unit for made for the consumer market ever sold. In 1967 the first attempts to stimulate the spinal cord began, and the first condition to treat was chronic pain, Shealy used a modern version of the Electreat to screen for patients and so transcutaneous stimulation was born (C. N. Shealy, Mortimer, & Reswick, 1967). In the same decade (1962) Lynn Bindman started applying electrical transcortical polarization in the rat model while recording evoked somatosensory potential, Bindman was capable to demonstrate cortical modulation of the sensorimotor area which was dependent on the applied polarity he also demonstrated the after effects in hyperexcitability as a result on the stimulation (figure 1.3).

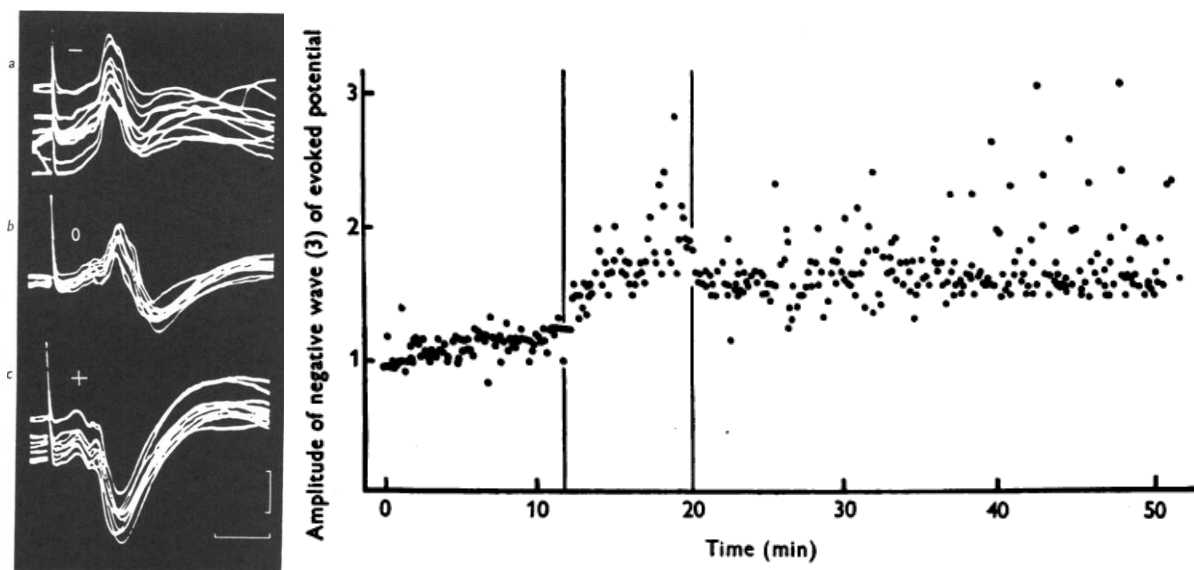


Figure 1. 3.Original traces from evoked sensory motor potentials recorded in the rat cortical model, the upper traces represents action potential elicited by a negative polarity, the traces in the middle are baseline potentials, traces in the bottom represents the effects of positive polarization to the cortex. Graph in the right demonstrating the after effects of cortical polarization, notice that after 10 minutes of stimulation the cortex remained exatible for 30 minutes after stimualtion. Adapted from Bindman, et al, 1962.

In 1969 the Russian neurologist Bechtereva published her work in chronic electrical stimulation and its effects on mental (cognitive) activity, Bechtereva concluded that electrical stimulation to the cortex was capable to modulate mental processes (Bechtereva, 1969), these

studies started to bring scientific attention to the fact that higher order cognitive functions are receptive to the effects of electrical stimulation, although at this point in time invasive and noninvasive electrical stimulation was becoming popular among neuroscientist, psychologist, neurologist, neurophysiologist, and neurosurgeons who oversaw these new methods as an alternatives to the treatment of brain and mental pathology, at the same time there were also discoveries in the field of neuro-psychopharmacology and gradually the “pharma-studies” started to cast a shadow on brain stimulation, as these new pharmacological agents offered sound basic data from animal and human studies. Nevertheless, advancements in brain stimulation led Melzack and Wall to the discovery of the “gate theory” presented in 1965, this theory described spinal mechanisms for the modulation of pain based on peripheral nerve stimulation, the gate theory proposed that a spinal segment corresponding to a peripheral nerve will open to allow pain transmission or close to inhibit the perception of pain, this gate would be modulated by the rate of firing corresponding to the spinal segment (Melzack & Wall, 1965). When the theory was applied for the control of chronic pain in clinical environments, it morphed to the so called transcutaneous electrical nerve stimulation or TENS, it marked the beginning of a commercialization period for this particular technique, moreover, it move up the concept of electricity used for therapeutic purposes.

In 1985 Anthony Barker from Sheffield University demonstrated evoked muscle potentials after magnetic induced electrical activity in the primary motor cortex in humans (Barker, Jalinous, & Freeston, 1985), Barker successfully demonstrated the mechanisms of noninvasive cortical stimulation by using external magnetic field pulses delivered through a cooper wired coiled, but more important, Barker was able to reignite the discussion about the use of electrical stimulation for human applications which lead to the re-discovery of tDCS. Michael

Nitsche in Gottingen University offered the first insight into the mechanism behind polarity dependent cortical modulation in humans (Nitsche, Liebetanz, Tergau, & Paulus, 2002) by delivering weak electrical currents applied transcranially, moreover, Nietsche replicated early studies showing the aftereffects of stimulation observed in the animal model but in human subjects (figure 1.4). The research supporting TMS after Barker’s publication and Nietsche work on tDCS propelled the generation of a new kind of scientist in neuromodulation, thanks to the principle that the brain can be modulated noninvasively and in a safe manner.

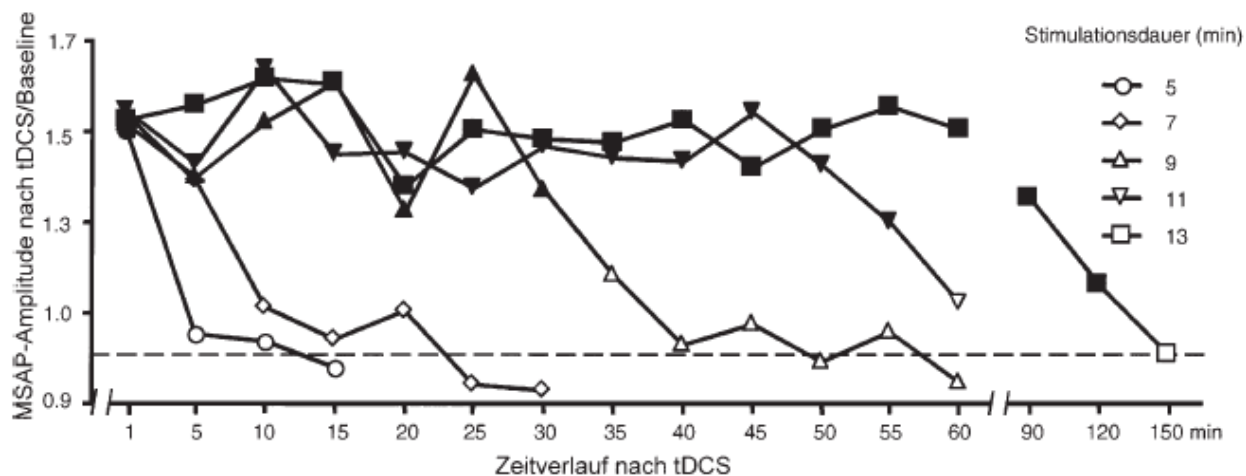


Figure 1. 4. Effects in the amplitude of motor evoked potentials. Observed effects in the amplitude of motor evoked potentials (MEP) after exposure to tDCS, the graph depicts five different stimulation durations, 13 minutes kept the modulation of the recorded MEP well beyond 60 minutes post stimulation, notice the similarities with the response curve observed in Bindman’s research (above). From Nietsche, et al, 2004.

Cranial electro therapy stimulation or cranioelctrical stimulation (CES) begun its development by the momentum offered in the late 1950’s, as some researchers started questioning the effects of small or weak currents when use in clinical conditions, in 1958 Gilyarowski published his book entitled “Electrosleep” (A Clinical Physiological Investigation), Medgiz-Moscow (1948) displaying the interest that clinical researchers in the Soviet Union already were investigating CES, it was not until the late 60’s than American researchers

presented their findings using CES in clinical trials (Taaks & Kugler, 1968). Joseph Ryan from the Veterans Administration Medical Center in Chicago presented clinical evidence of CES exposure in individuals with sleep and anxiety disorders (Ryan & Souheaver, 1976, 1977) Ryan and collaborators suggested that the electrical current reached somewhat the pons, medulla, and midbrain structures, affecting the reticular ascending activation system, thus ameliorating sleep disturbances and concomitant anxiety. After a series of compelling clinical data the FDA approved the use of electrotherapy for the treatment of insomnia, anxiety, and depression and in 1978 it change the nomenclature to CES, A recently revised annotated bibliography of CES research summarized 126 human studies, 29 animal studies, and 31 review articles in the English language literature (Kirsch, 1999), important to mentioned is the fact that most of the studies focused in clinical results and just a minority of them explore basic biological explanations in regards CES properties, moreover, no studies presented mechanistic attributes of CES in relationship to neurophysiological processes. CES has been morphing as the attributes of the stimulation change depending on the parameters, thus as a basic form of alternating current (AC), CES can be delivered by sine waves or pulses and pulses can be monophasic or biphasic in relationship to the offset, they can also be presented in a fixed frequency range or randomly alternating within an established range. This research work will present the analyses related to tPCS neuromodulation, tPCS a form of CES with AC properties but with distinctive characteristics that might separate this technique from the rest of CES approaches.

1.4 Research Problem

Could different parameters of tPCS applications, would lead to differential changes in the neurophysiology as measured by qEEG? And if so, what would be the impact of these changes on cognitive and behavioral tasks?

TPCS is also known as tACS, or CES. These variations in nomenclature echoes the issues involved in the specific characterization of the principles governing tPCS mechanisms of action, yet tPCS has FDA clearance for use in the treatment of depression, insomnia and anxiety related symptoms – and it has shown to be a useful technique to modulate subcortical neural circuits as revealed by current modeling simulations. It seems irrational to believe that a technique that has been approved for its use in humans still lacking the essential knowledge in how to control the properties of such current delivery and how these parameters impact the physiology and function.

In this study, the researcher aims to address the gaps in the investigation of this technique – including characterization of optimal parameters to induce modulation of cortical electrical activity, moreover, it also explores its role as potential modulator of cognition and behavior, throughout multiple evaluations of its parameters and its effects on mechanisms of action. These will be indexed by changes in processed EEG calculations, and cognitive testing. Currently, we are in a period where the establishment of optimal stimulation parameters is necessary for reliable cortical-subcortical neuromodulation, also by understanding the dynamics of the proposed parameters; we will be able to correlate its mechanisms of function for possible application of this technique to cognitive modulation applied in pathological conditions, this research presents evidence of reliable testing using sophisticated methods so we can understand better the role of tPCS as a NIBS technique, more important this evidence confirms the qualities that position tPCS as a well defined tool for neuromodulation.

1.5 Objective and Aims

The main objective of this research was to generate knowledge regarding tPCS mechanistic properties and evaluate these parameter properties for application in cognitive and

behavioral modulation/enhancement, including potential strategies to develop research methodologies for future clinical protocols. Given that tPCS has a direct effect on cortical areas; modulation of subcortical neural networks can be established via different stimulation parameters/mechanisms. Therefore the evaluation of tPCS by QEEG approach in different experimental settings provided valuable information on how this technique modulates cortical networks.

The specific aims of this study were to;

Aim 1: The initial aim of this investigation was to determine which tPCS parameters; frequencies and current intensity, elicited a measurable qEEG change in the power and mean frequency of the EEG bands. *The hypothesis established that low-frequency, low-current intensity, and short time of application will not generate much change in the qEEG analysis, while higher frequencies will evoke EEG band shifts on the qEEG power and mean frequency analysis.*

Aim 2: Using optimal parameters of stimulation defined in aim 1, we then evaluated the impact that tPCS had on the neuropsychological domains; this was estimated by evaluating cognitive functioning and performance in the research participants using a specific set of neurocognitive assessments.

Aim 3: Using optimal parameters of stimulation defined in aim 1, we also investigated the relationship between tPCS modulation and changes within physiological responses during neurocognitive testing. To do this, heart rate variability and the electrodermal skin response was measured as markers of stress response and tolerability.

1.5 Thesis structure

This thesis has been organized in a manner the reader can track the development of this research. This work is presented in six chapters as follows;

Chapter 1: introduces some basic background and history, it also offers technical definitions and presents the problem and propositions.

Chapter 2: reviews relevant literature, and categorizes the seven themes that presents as relevant for the development of the experiments described in this thesis.

Chapter 3: describes the action research methodology used to develop each experiment that has an impact on the key theories.

Chapter 4: reports findings in the context of each experiment researched.

Chapter 5: discusses the generalized theory in the form of the four presented experiments, the discussion also recognizes the implications for the conceptualization of new theories to be explored and are inherently tied to the findings exposed in this research.

Chapter 6: presents the conclusion and considers issues of validity in the form of a case presentation.

2. LITERATURE REVIEW

2.1 Introduction

This chapter reviews significant, up to date literature concerning principles of NIBS and tPCS in relation to neurophysiology and cognitive and behavioral functioning. The basis of electrical stimulation, functional anatomy and cognition are also presented. The chapter provides an overview of the used physiological measurements and evaluations that contributed to differentiate the mechanisms behind tPCS. Cognitive assessment methods used in relation to tPCS characterization are discussed in addition to focusing upon papers that have studied similar NIBS interventions. The effect that tPCS has on; electrical cortical activity, cognitive and behavioral functioning will be addressed. The rationale for the use of qEEG as a main outcome will be explored and both negative and positive aspects of the study population will be discussed. The chapter will conclude with a discussion about the success of the tPCS intervention in this research and the scope for further clinical studies will be undertaken.

It is important to mention that although there have been few publications investigating specifically tPCS, most of the presented literature for this technique are manuscripts already published or in revision authored by the investigator presenting this doctoral thesis. Therefore a number of manuscripts presented in this review will describe or use the nonspecific CES or tACS nomenclature, however, as tACS is physically different form tPCS (sine waves vs. pulses), it will be only presented to demonstrate these dissimilarities between the two of them.

It is the purpose of this chapter to introduce and guide the reader to the understanding of many of the elements and concepts discussed in this research work, to cover all of the material is

a difficult task, however, this chapter is organized in a manner that allows the progression of concepts in a fluent approach.

2.2 Principles of Electrical Stimulation

When electrical currents are delivered to the nervous system to elicit or inhibit neural activity, two things can happen: first the current creates a potential field that can alter the state of the voltage-gated ion channels, proteins that are embedded in the membranes of neural elements; and second, electrochemical reactions occur at the electrode–tissue interface. Altering the state of voltage-gated ion channels can initiate or suppress a propagated action potential, which, in turn, affects the release of neurotransmitter at the terminal end of the axon. Uncontrolled electrochemical reactions, at the electrode-tissue interface, can cause damage to the electrode or injury to the target tissues. *From Mortimer and Narendra in Neuromodulation* (Krames, et al., 2009). This paragraph serves as a perfect introduction to understand the interaction between electrical energy and the neural tissue. Due to the nature of how the flows of electrons move from one electrode to another, it modifies the physiology of any excitable tissue that is under the exposure of this flow. This flow of electrons is called *current* and for these electrons to move they will need a dynamic force in order to keep this electrons motion, this force or “pressure” is called electromotive force and represents the *voltage*.

Electrical current is measured in amperes, which is the total amount of charge that moves through the electrodes over time. To better understand this basic concept of electricity we can rely in the presentation of the Ohm’s law that states that current is the same as the voltage divided by the offered tissue resistance: $\mathbf{I} = (\mathbf{V}/\mathbf{R})$, by having this principle on mind, one can predict that by increasing the voltage and assuming resistance does not increase, then the current

will increase as a consequence of it, this is an important principle because it plays a significant role when designing electrode montage for noninvasive stimulation.

Current density refers to the corresponding amount of current delivered to a specific brain area, if the current intensity increase a damage can be induced to the neural components, this issue is an important limitation of electrical brain stimulation, as higher doses are toxic to neural cells. Based in Ohm's law, the electrical system can operates under two conditions; 1) constant current or 2) constant voltage, both principles can be applied in brain stimulation and each of them will lead to different responses within the neural system.

The total amount of electrical power that will move throughout the system can be calculated easily by multiplying volts by amperes, the product is called a *watt* ($V \cdot A$). The resistance that the current encounters during its motion is measured in *ohms* and this parameter is of crucial importance when calculating current doses, as resistance limits the conduction or the total impact of the current over the desire targeted tissue.

Resistance and conductivity are inversely related, as poor conductors' present high resistance, while good conductors show low resistance. Depending on physic and chemical structural properties materials have different capacities to conduct and resist. Characteristically, copper is an example of a good conductor with low resistance, whereas rubber is a poor conductor presenting high resistance. In brain stimulation, the skull is a terrible conductor with high resistance. Different brain tissues such as neurons and spinal fluid are generally excellent conductors with low resistance (Higgins & George, 2008).

The interface between the stimulation apparatus and the cranial structure is called *electrode*, the electrode diffuses the electrical energy by accomplishing the connection through

the two opposite poles of the electrical source. The electrical current flows from the positive polarity of the electrical source to the negative polarity while the negative charges (electrons) flows in the opposite direction. The processes occurring at the *cathode*, are defined as those where reduction of species in an electrolyte solution or environment occur as electrons are transferred from the electrode to the electrolyte. The processes occurring at the *anode*, are defined as those where oxidation of species in the electrolyte occur as electrons are transferred to the electrode.

The understanding of how the current is delivered by either anodic or cathodic means is of paramount importance, by facilitating the increase positive valance or electron removal, the neuron membrane potential will be prone to depolarize, while by decreasing the positive balance or electrode gain the effect will be the opposite, in this case repolarization of the membrane potential. This principles of action are the cornerstone for applying NIBS and by selecting the type of technique to be used, for instance in tDCS the polarity will define the desired physiological effect as the current will followed a pre-established electrode montage with the purpose to either facilitate or inhibit neural processes, this is not the case for any form of alternating current, as the flow of electrons will present a dynamic back and forward motion, so the electrode montage will take into account just the structure to be affected without focusing in polarity dependent effects, figure 2.1 shows the current motion present in direct current and alternate current stimulation.

To summarize, we can describe the stimulus characteristics as the electrical charge applied to effect stimulation of neural excitable tissue in a temporal manner by the current. The unit of measurement for the applied charge, a voltage or current pulse, is defined by the duration of it (commonly known aspulse width), amplitude (in Volts or Amperes) and finally pulse

morphology (quadratic, rectangular, triangular, or sinusoidal). The repetition rate of the individual pulses is the stimulus frequency or pulse rate when alternate current is being applied.

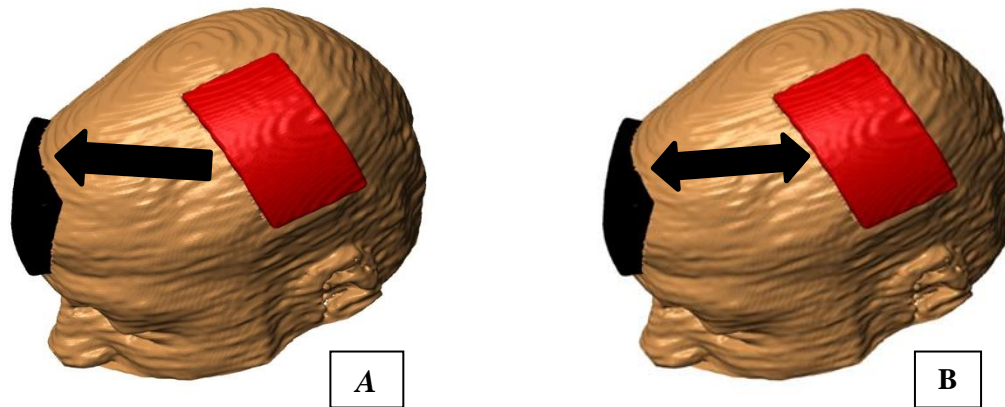


Figure 2. 1. Schematic representation of current flow in a direct current montage (A), notice the directionality of the flow from anode (red electrode) to the cathode (black electrode). Alternating current presents a bidirectional flow that keeps moving back and forward between the anode and cathode (B).

2.3 Neurophysiology

There are three main ions that are separated across a nerve membrane at rest. The concentration of sodium (Na^+) and chloride (Cl^-) is considerably higher in the extracellular space than in the intracellular space, while potassium (K^+) is highly concentrated on the inside of the neuronal membrane when compared to the extracellular space. The resting potential of the membrane is about -70 mV, inside with respect to outside, which is close to the ionic equilibrium potential for both K^+ and Cl^- , this value is determined by the difference in ion concentration between the two sides of the neuronal membrane. K^+ and Cl^- concentrations are the two electro-reactive ions who determine the resting potential across the neuron membrane. The resting nerve membrane is poorly permeable to Na^+ and the Na^+ equilibrium potential is about -55 mV, which drives the inward current flow (intracellular flow) during the action potential (figure 2.2).

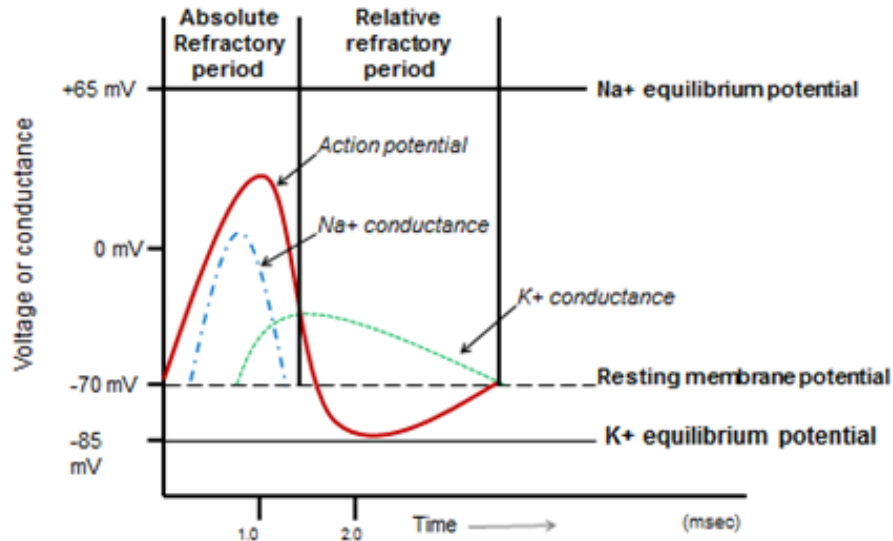


Figure 2. 2. Ionic equilibrium for the generation of an action potential, during the absolute refractory period there is an inward flow of Na^+ while K^+ moves outwards facilitating the depolarization of the neuronal membrane.

Depolarization is the term that refers to the membrane potential when is less negative. The *hyperpolarization* membrane state makes the membrane potential more negative. An *Inward current* is the flow of positive charge (Na^+) into the cell. The inward current drives the depolarization of the membrane potential. *Action potential* is a characteristic property of excitable cells that consist of a rapid depolarization, or upstroke, followed by repolarization of the membrane potential. Actions potentials have stereotypical size and shape, and propagate all along the cell membrane. Action potentials have the particular characteristic to be all-or-none, which means that the inward current should carry enough Na^+ to the point to reach the threshold for membrane depolarization. Threshold is the membrane potential at which the action potential is inevitable. Inward current depolarizes the membrane. If the inward current is not sufficient to depolarize the membrane to threshold, it does not produce an action potential. If the inward current depolarizes the membrane to threshold, it produces an action potential. As mentioned above the electro-reactive ions represent the basis for the neuronal action potential, where the

resting membrane potential is approximately -70 mV and is the result of the high resting conductance to K^+ , which drives the membrane potential toward K^+ equilibrium. At rest, the Na^+ channels are closed and Na^+ conductance is low. It is believed that externally applied stimulation via attached electrodes to the scalp, promotes subthreshold modulations at the membrane level due the weakness of the current intensity. This subthreshold modulation should be affecting the membrane potential trough the ion channels (figure 2.3).

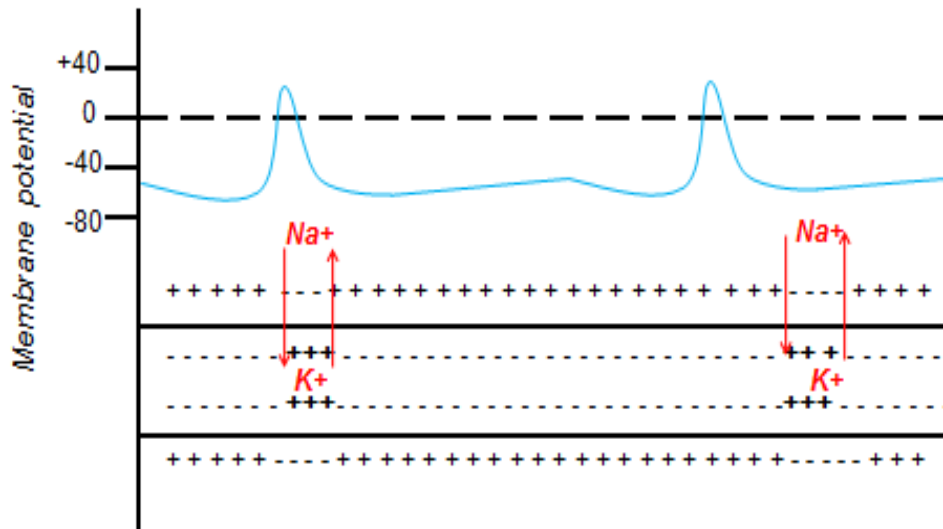


Figure 2. 3. Intra and extracellular membrane polarity changes, Na^+ flows from the outside to the intracellular space while K^+ moves in the opposite direction thanks to the transmembrane conductance properties of excitable cells.

Voltage-gated ion channels are a class of transmembrane proteins that are activated by changes in electrical potential difference across the cell membrane (Armstrong & Hille, 1998). Voltage-gated sodium ion channels (Nav) can have three possible states: closed-activatable, activated (open and conducting), and closed-inactivatable (Gregerson, 2003). The opening of Nav is a stochastic process that is potential-dependent, meaning that as the transmembrane potential increases, the probability increases for a resting channel to transition to a conduction state. In the conduction state, each channel is acting as a miniature current source (Doyle, 2004).

This randomly determined *Nav* process takes special significance when we considered how it will affect the firing rate. The injected current to the tissue can determine the rate of neuronal firing, for instance, visual stimulation at 10Hz cycles promotes the generation of alpha oscillations recorded at the occipital pole, meaning that the rhythmicity of the visual stimuli can entrain the background rhythm captured in the visual cortex, however, when a random stimulation is presented the recorded signal does not follow the randomness, but rather increases the power of the ongoing frequency that is innate to the neuron at that particular time, this phenomena will be discussed latter in the discussion once the results are presented. When the action potential travels along the fiber, ending in an excitatory synapse, an excitatory postsynaptic potential (*EPSP*) occurs in the following postsynaptic neuron.

The electrochemical properties of the synapsis dictates the response of the postsynaptic neuron, if an action potential travels along the fiber ending in an inhibitory synapse, the hyperpolarization will occur, representing an inhibitory postsynaptic potential (*IPSP*), the net result for excitation or inhibition depends in the electro-chemical nature of the synapsis, neurotransmitters with excitatory properties such as glutamate generates depolarization, while GABAergic neurons produces inhibition if the action potential reaches their presynaptic membrane. The Excitation/inhibition of neurons by neurotransmitters is dependent on the protein segments extend out of the membrane and serving as a receptor site. Ionotropic receptors directly alter the conductance of the ion channel when bound to a neurotransmitter, thus GABAergic receptors increase Cl^- conductance facilitating IPSPs, while N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) receptors are permeable to Na^+ and calcium (Ca^{+}) both ions will facilitate excitatory synaptic transmission.

If two action potentials travel along the same fiber with a short interval, there will be a summation of *EPSPs* triggering a stronger response on the postsynaptic neuron, if this summation of potentials preserves overtime a state of synaptic plasticity will take place.

2.3 Neuroplasticity

Neuroplasticity represents the functional component of learning and memory and is a mediator of responses to neuronal cell attrition and injury (compensatory plasticity). It is a dynamic process in reaction to neuronal activity, growth and development, death, and genesis, which involves modulation of structural and functional processes of axons, dendrites, and synapses.

The main attribute of neuroplasticity relay on the synapsis, Synaptic plasticity is the capability of synapsis reorganization by means of neurotransmitter diffusion, between two neurons in order to change the strength or stability of the connection. There are several underlying mechanisms that cooperate to achieve synaptic plasticity, including changes in the quantity of neurotransmitter released into a synapse and changes in how effectively cells respond to those neurotransmitters. Synaptic plasticity is activity-dependent modification of synapses is a powerful mechanism for shaping and modifying the response properties of neurons, and provides the basis for most models of learning, memory, and development of neural circuitry. Synaptic plasticity as elemental component of communication is also the main target for reorganization as a result of activity dependent strengthening. The spatial extent of such large-scale reorganization suggested that multiple mechanisms account for these changes (Mogilner, 1993), including:

1. Simple computational changes in the relative weights of excitatory and inhibitory inputs to a predefined neural matrix

2. Axonal sprouting
3. Changes in synaptic size, number, and morphology
4. Reorganization at the subcortical level.

These changes in synaptic strength must occur across multiple synapses and coordinated appropriately, so the levels of synaptic activity in a neural circuit promote the growth or shrinkage of connections in a functional manner. The most studied model of plasticity is the *Hebbian model* described by Donald Hebb and developed at McGill University, the *Hebbian Plasticity* model states that synapses effective at evoking a response should grow stronger, over time Hebbian plasticity has come to mean any long-lasting form of synaptic modification (strengthening or weakening) that synapse specific and depends on correlations between pre and postsynaptic firing. The Hebbian model is positive-feedback process because effective synapses are strengthened, making them even more effective, and ineffective synapses are weakened, making them less so, this creates instability at postsynaptic firing rates. Two rules for synaptic modification are proposed in this model 1) *Wire together fire together* and 2) *Neurons Out of synchrony lose their link* (Hebb, 2005). Based in Hebb's model, long term potentiation (LTP) facilitates the strengthening of the synapsis under the influence of glutamatergic receptors (NMDA and AMPA receptors) it is also modulates voltage-gated ion channels for Ca⁺ and Mg⁺, these mechanisms are of special considerations for NIBS as LTP can be affected by electrical stimulation. Contrary to LTP is long term synaptic depression (LTD) which validates the concept of neurons firing out of synchrony promoting the weakness of the connection, LTD does not seem to be mediated by glutamatergic receptors, on the contrary, synapsis firing out of synchrony loss their AMPA receptors.

Long term potentiation and long term depression are the most studied substrates of synaptic plasticity. Synaptic strengthening, which requires activation of pre- and postsynaptic elements underlies the phenomenon of LTP as a model of memory formation, and which is associated with synapse dynamics including formation and removal of synapses and changes in synapse morphology (Chang & Greenough, 1984). Signals of plasticity include intraneuronal (anterograde and retrograde), interneuronal (transsynaptic and extra/parasympaptic) as well as intercellular signaling through glia (Cotman & Nieto-Sampedro, 1984). Those neuronal systems playing a crucial role in higher brain functions (e.g. learning, memory, cognition) such as hippocampus, neocortical association areas, and the cholinergic basal forebrain neurons, retain a high degree of structural plasticity throughout life (Arendt, 2004). The many factors acting in favor or against synaptic plasticity are many and at this point we still trying to understand those mechanisms, molecular principles play an important role and to describe all of them is a subject for volumes about the research that has been identifying each of these molecular components, we can mention the most significant components for synaptic plasticity as; 1) Long Term Potentiation/depression driven by the calcium/calmodulin protein complex activity, 2) Glutamatergic receptors activation (NMDA/AMPA), 3) Synaptic efficacy and remodeling (mediated by cell adhesion molecules), 4) Synaptogenesis and neuritogenesis (axonal sprouting and dendritic remodeling under the influence of trophic factors), and 6) neurogenesis at hippocampal level by gene activation.

As these biological mechanisms of synaptic plasticity favors circuit reorganization for functional outcome, if not properly regulated, the very same mechanisms can start a process of maladaptive plasticity which is the common denominator for conditions such as epilepsy, autism, or chronic pain. Neuroplasticity is seen a process that happens after damage, however, plasticity

has been part of every individual from the very moment of conception. Developmental plasticity is the main force behind CNS specialization. Components of developmental plasticity include; Growth factors genes and homogenes, the first set of activated genes provide the encoding to favors cellular energy needed for development, while the second set of genes facilitate the spatial and temporal expression of growth factor genes. Some of these expressed growth factors are; transforming growth factor beta 1 (TGF- β), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF). Transcription Factors which act as “interactive factors” for genetic to proteic expression is driven by transcription, transcription is a process by which the DNA sequence is copied into an RNA sequence with the purpose to enable protein encoding sensible to the effects of the above trophic factors.

The orchestration of all these biological properties give rise to the formation of learning, memory, and motor function which all together are the generators for behavioral output, figure 2.4 summarize in a diagram all of these components in a feedback-forward organization that promotes self-modulation of each of these units within the system of neuroplasticity and reorganization based in the developmental model. To better appreciate how plasticity occurs from a biological perspective, one must pay attention to the hierarchical sequence of activation, from gene and RNA activity which in turn promotes protein and receptor expression, to morphological changes observed by dendritic arborization and spine formation with synaptic consolidation, these morphological changes are in response to functional modifications where neural networks are the final recipients benefiting from this organizational structure.

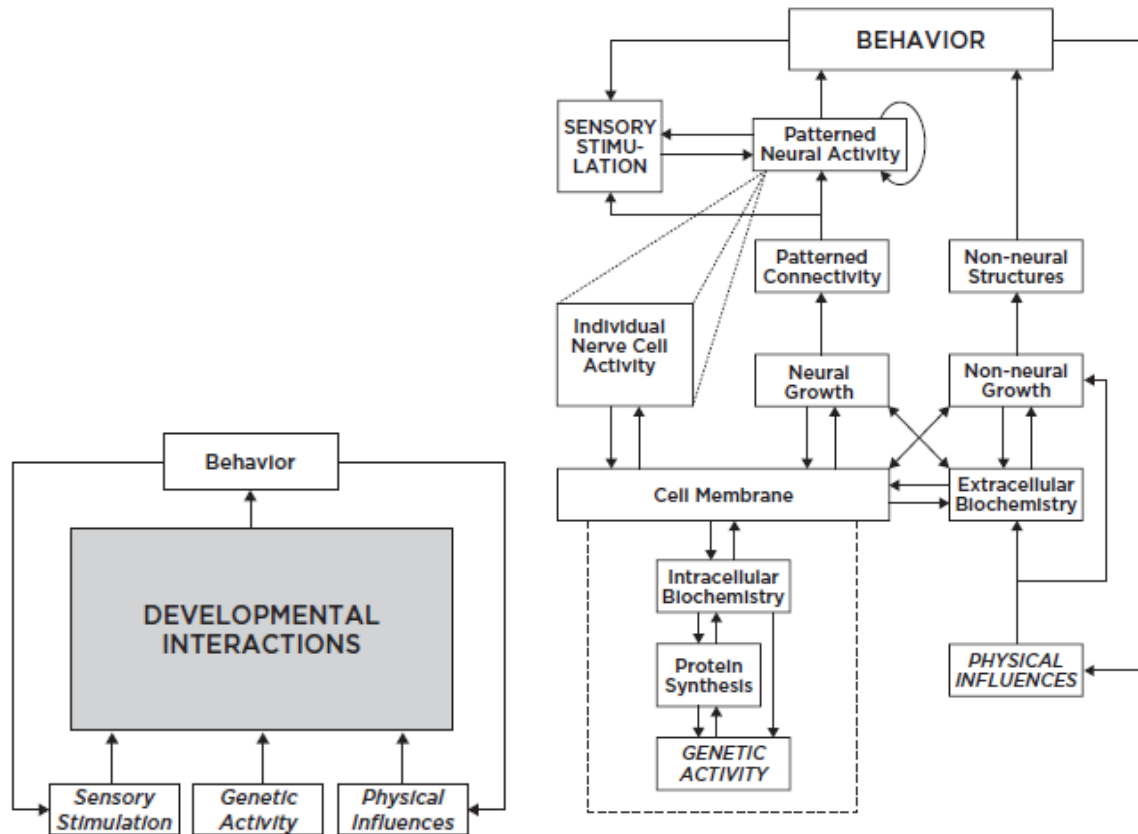


Figure 2. 4. Neurodevelopmental model of plasticity involving different biological and molecular systems. From(Johnston & Edwards, 2002)

Different models of plasticity have been proposed thanks to the advancements of computational neuroscience. Briefly, some of these models are presented. The BMC developed by Elie Bienenstock, Leon Cooper, and Paul Munro states that pre and postsynaptic activity evokes LTP when the postsynaptic firing rate is higher than a threshold value, while LTD is kept lower. In order to stabilize this model, the threshold must shift as a function of the average postsynaptic firing rate and the opposite will occur by reducing the postsynaptic activity decreasing LTP threshold and increases the LTD threshold. The opposite applies to the increase in postsynaptic activity (Bienenstock, Cooper, & Munro, 1982). It is imperative to understand that it has long been known that presynaptic activity that precedes postsynaptic firing or depolarization can induce LTP, whereas reversing this temporal order causes LTD. Another two

models based in these assumptions are *Synaptic scaling* stating a multiplicative post-synaptic strength as a result of scalar firing over the same synapsis and *Spike-taming dependent plasticity* where dynamic NMDA receptor activity interactions and timing of depolarization and propagation in the postsynaptic dendrites and neurons facilitates the strength of the connection (Cain, 2001). Another model of synaptic plasticity that explains the process of synaptic optimization is *Synaptic redistribution* where a synapse can be strengthened post-synaptically by increasing the number or efficacy of receptor channels or pre-synaptically by increasing the probability amount of transmitter release. This model describes morpho-molecular changes occurring at pre and post membrane level, these changes not only increase the capability for connection, but it also decrease the associated energetic cost associated with firing by spreading the receptors all along the postsynaptic membrane. Figure 2.5 summarize the mechanisms involved in the process of plasticity. It presents the backbone of biological factors affecting the neurons modifications, these changes can occur as a result of experience or as a consequence of neuronal insult. By understanding neuroplasticity we can comprehend better how NIBS techniques can either inhibit or facilitate cognition.

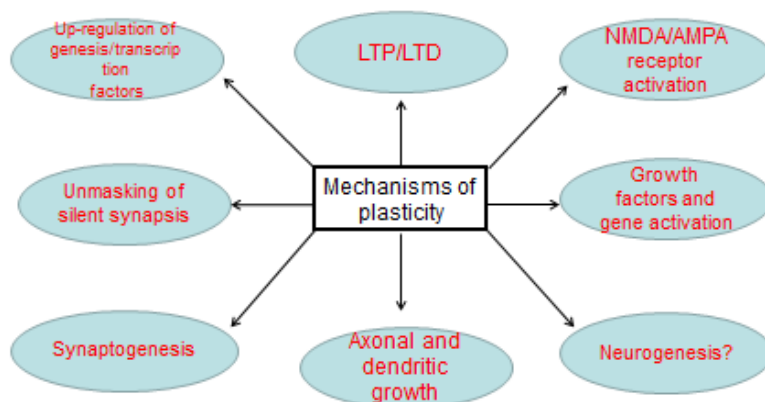


Figure 2. 5. Biological mechanism affecting neuroplasticity processes. Neurogenesis has been demonstrated in animal models, it is thought the same might happen in human brains especially in temporal lobe structures.

2.4 Physiological basis of Electroencephalogram (EEG)

The EEG is a visible record of the amplified electrical activity generated by action potentials of the cortical neurons. The activity recorded are the summation of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs respectively) produced by the pyramidal layer of the cerebral cortex. The electrode on the surface of the neuron at rest is at 50-80 mV positive to an electrode inside the axon. The semipermeable membrane of the neurons keeps sodium ions (Na⁺) outside and retains potassium ions (K⁺) inside. This equilibrium is maintained by the sodium-potassium pump which by gradient in voltage facilitates the Na⁺ concentration in the outside of the cell. The flow of ions along the axon membrane changes the threshold for firing, and the action potentials occur (depolarization), then ions come back to the rest baseline concentration (repolarization) until another stimulus promotes the shift in the ions concentration gradient (Olejniczak, 2006).

The potentials of the neurons change synchronously, and they generate rhythms of distinctive characteristics. The thalamus is considered the subcortical structure that originates the EEG activity, and the waves captured in an electroencephalography system are the product of the potential difference between two electrodes (Tyner, Knott, & Mayer, 1983).

The first time an EEG was recorded happened in 1924 when Hans Berger placed a pair of electrodes in the frontal and back regions of the skull. He was able to see synchronized waves that showed variability in two dimensions: time and amplitude. Time is measured in Hz and express as frequency, and amplitude is measured in voltage microvolts (μV) (Jung & Berger, 1979). As a form to localized cortical areas in the brain, a simplified coordinates system was developed in order to get topographic a representation of these cortical structures. There is an

international electrode placement that recognizes the exact localization of the electrodes over the scalp (figure 2.6). The names of the electrodes are the first letters associated with the areas where the electrode is placed, and the number indicating the lateralization and placement within the areas. The 10/20 electrode placement system use anatomical references to standardized the measurements among individuals, the *nasion* in the anterior portion of the head representing the point of junction between the nasal bridge and the beginning of the forehead, serves a point of measurement ending in the *inion*, which is most protuberant point of the occipital bone in the posterior part of the head. Laterally the preauricular points are used to draw a line that crosses the head from ear to ear; once these lines are measured a set of 10% or 20% portions make the coordinates for topographic brain representation. The electrodes are made of silver-silver chloride that can measure slow shift of potentials (range from 0.1 to 70 Hz). The metal electrode placed in a conducting solution forms an electric double layer between the solution and the metal, where the ions flow and can be translate in a graphic form (Tyner, et al., 1983).

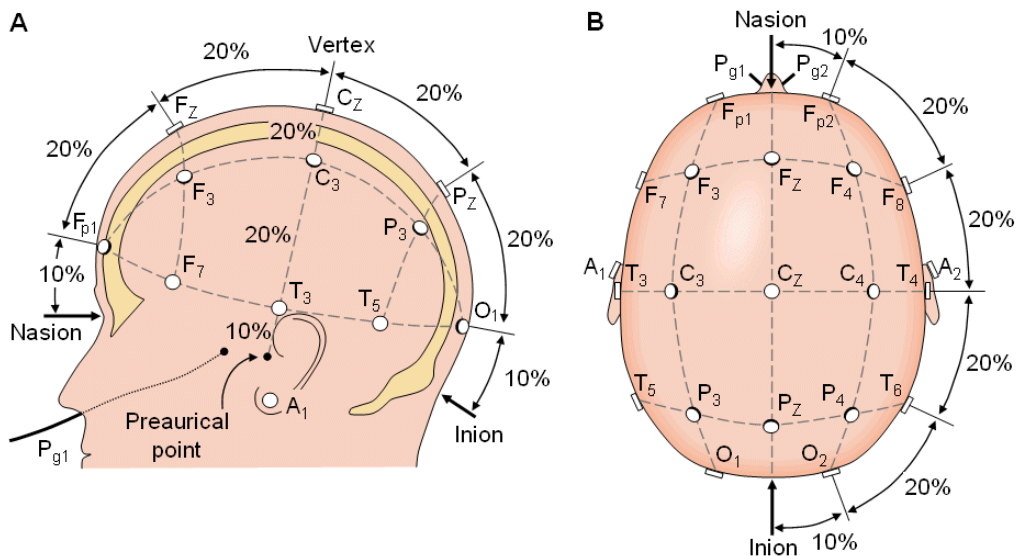


Figure 2. 6. International 10/20 measuring system for EEG recording. Anatomical references are shown. From <http://www.bem.fi/book/13/13.htm>

In traditional clinical electroencephalography four frequencies bands can be observed in human recordings. *Alpha* is the most prominent signal frequency in a healthy adult it ranges from 8-13 Hz and is well localized in the posterior regions during eyes closed. Alpha is a synchronized oscillation that represents the prototypic frequency for neural wellness, it has been used as an EEG marker that can be quantified to as to elucidate the degree of brain pathology (Anghinah et al., 2000). Lower amplitude, high frequency waves that range from 13-20 Hz area are called *Beta* waves and mostly represent a state of local network activation, beta activity can be observed during movement planation and performance or other higher mental process. Slower frequencies with high amplitude ranging within 4-7 Hz domain are called *Theta* and this activity is present during drowsiness and should not be present in a healthy awake adult, however, during high cognitive demands theta is present in some temporal and prefrontal areas accompanying the occurrence of higher frequencies that usually are above the beta range. The activity below 4 Hz is called *Delta* and is an activity present during some stages of sleep (Lopes da Silva, 1991) and states of deep brain damage, figure 2.7 illustrates the main frequencies described above.

These EEG frequencies in a healthy adult modify during changes in consciousness and during cognitive engagement, some frequencies tend to be more prominent in specific areas of the brain (e.g. frontal and parietal lobes during arithmetical processing). Alpha is an activity that is present in a healthy, relaxed state in adults having their eyes closed, it will be more prominent in the occipital area and the amplitude of the alpha oscillation will decrease when visual stimulation is presented. Cognitive task will engage the activity of specific networks and will switch to a higher frequency (Beta) and lower amplitude. Sleep is a complex process in which there are several changes in frequencies of the EEG (Tyner et al., 1989).

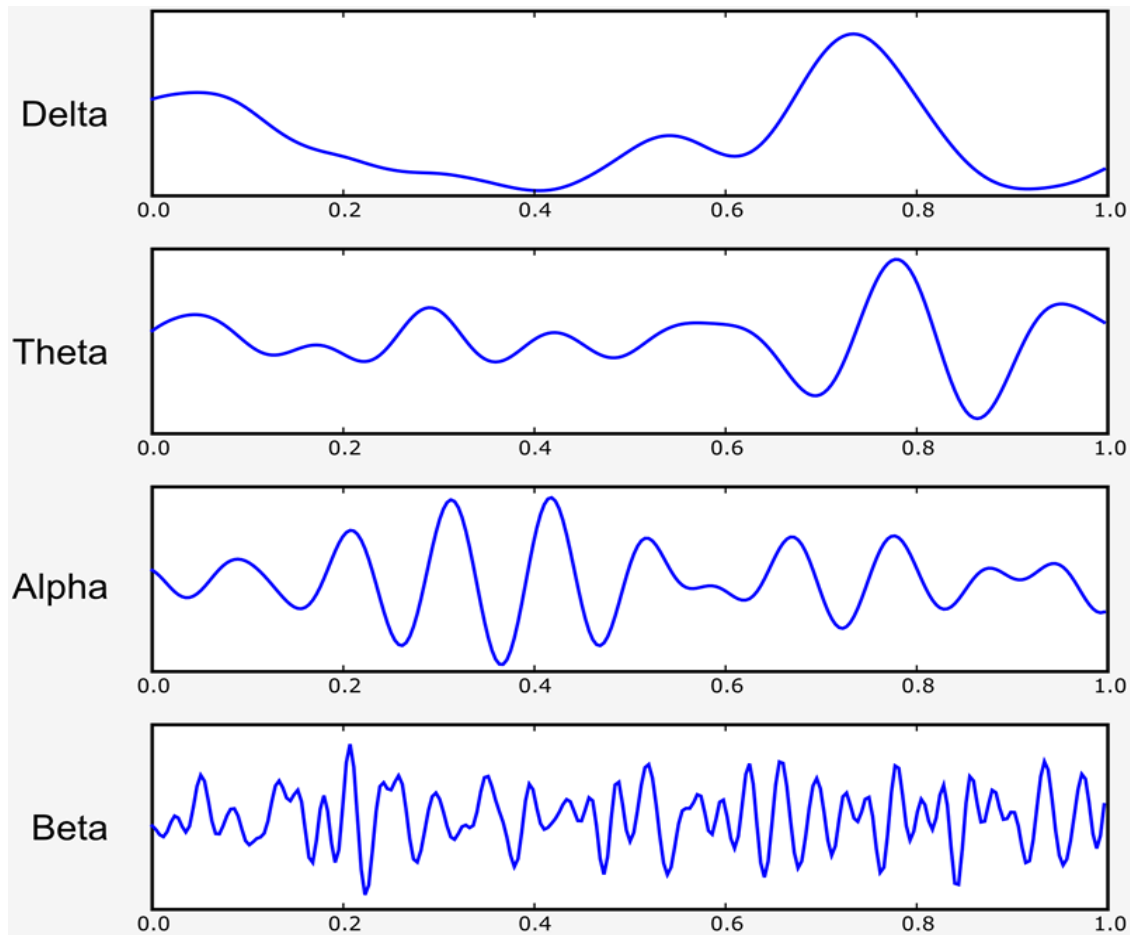


Figure 2. 7. Frequency bands clinically relevant to electroencephalographic frequency bands. The more distinctive features among these frequencies are the differences in amplitude in mV and cycles per second in Hz.

The EEG is currently used to identify abnormal brain activity related to neurological conditions. The most common disorder evaluated using the EEG is epilepsy, as is recognized by producing abnormal cortical electrical discharges that cause seizures, and abnormal activity can be recorded in an EEG as fast high voltage waves called spikes followed by slow waves called spike and slow wave activity (Adebimpe, Aarabi, Bourel-Ponchel, Mahmoudzadeh, & Wallois, 2015).

The EEG is a very valuable tool to evaluate head injuries, encephalopathies, encephalitis, brain tumors, and strokes, sleep disorder, and lately is widely use to assess cognitive functions.

The EEG device magnifies the recorded potential between an active EEG source and the reference electrode. This differential amplification of the signal serves to eliminate common artifacts while amplifying the difference between two EEG inputs.

Presently the EEG machines are digital and transfer the continuous EEG signals into a binary system. The use of filters is essential as they function by blocking frequency components in a signal and passing the original signal minus these suppressed components to the output. A digital filter performs a mathematical operation on the signal. The functional filters as lowpass, highpass, and bandpass are distinct by their responses to the individual frequency components that constitute the input signal (Valencia, Martinerie, Dupont, & Chavez, 2008).

The processing of the EEG to a single numerical trend indicator (EEG parameter or EEG variable) for monitoring cortical electrical activity can be divided into three steps:

1. amplification and filtering of the electrical biosignal received by at least two electrodes mounted on the scalp or other parts of the head;
2. digitization of the amplified signal, generating a discrete numerical time series;
3. partitioning of the numerical time series into segments by grouping adjacent numbers and, using an algorithm, projecting each of these on to a number (the value of the EEG parameter as defined by the algorithm for that time segment).

Crucial for the analog to digital conversion process is the sampling frequency, the inverse of which is the equidistant spacing of the digitized amplitude, the smaller of the equidistant spacing, the more samples will be available and the better the original signal can be reconstructed (Schwilden, 2006). The localization of the electrodes is essential to read and interpretation of the

EEG data. The montages are different schemes to measure the EEG signal by interpolating the set of electrodes to a specific reference on the scalp or outside of the cranium area, come examples of these montages are; linked ear reference montage, bipolar montage or average montage

2.5 Quantitative Analysis of the EEG (QEEG)

The American Academy of Neurology defines the QEEG “the mathematical processing of digitally recorded EEG in order to highlight specific waveform components, transform the EEG into a format or domain that elucidates relevant information, or associate numerical results with the EEG data for subsequent review or comparison” (Nuwer, 1997) Modern technology gives us the availability to use the computerized system from the signal analysis. The EEG signal can be analyzed in a very sophisticated way to provide detail information. The main motivation of the development of the QEEG is based on the need of more objective information about brain activity and connectivity. It link human research into computational models of neurobiological and neurophysiological process and offers an opportunity for cross-species comparison.

In the past QEEG was based on frequency related analysis, the signal was decomposed into frequency bands, and the power spectrum was obtained (Hughes & John, 1999). Lately, more novel techniques have been implemented to assess comprehensively the brain activity and connectivity. Linear methods and nonlinear methods as theory based time series has been implemented. The supremacy of EEG/QEEG is the high temporal resolution; it can capture the rapid brain dynamics. In essence, the analysis of the EEG is the analysis of time series. In general, one may classify time series by the following properties: random versus deterministic, stationary versus non-stationary and linear versus non-linear.

The Fourier analysis theorem is used for this purpose. The Fourier theorem states that a signal (X) within time limit (t) from 0 to T^{11} can be decomposed into a set of simple sinusoidal functions, called Fourier series. These parameters can be represented in a form of amplitude spectra, power spectra and phase spectra, Raw EEG data consist of a mixture of many waves at different frequencies(1-70Hz). FFT provides a numerical value by decomposing these waves into voltage for each frequency point which provides *the power spectrum* (Kropotov, 2010). The basis of spectral analysis is a theorem stating that any function in time can be thought of as a superposition of sinus waves of different frequencies, and it can be calculated under the following equation;

$$eeg(t) = \sum_{\text{all frequencies } fi} A_{fi} \sin(2\pi f_i t + \delta_i)$$

Where coefficients of amplitudes (A_{fi}) and phases (δ_i) consider the square of the amplitudes (A^2) as a function of the frequency fi , this function is usually said to be the power spectrum of $eeg(t)$ because it gives a measure of how strongly a certain frequency fi contributes to the signal. That is, instead of representing the signal $eeg(t)$ by N data points $eeg(t_1), \dots, eeg(t_N)$, it is now represented by the amplitudes A_{fi} and phases δ_i at exactly $N/2$ frequency. Hence N original numbers (the signal data) are transformed into N transformed numbers (A_{fi}, δ_i). This transformation is called Fourier transformation. *Power* is the square of the EEG amplitude, mathematically it indicates the strength of the signal at the given frequency or frequency interval.

Typical data derived from QEEG;

- *Spectral analysis*: frequency composition over a given period

- *Absolute* and *relative* amplitude within a frequency range or at specific channels:
 $\mu\text{V}^2/\text{cycle}/\text{second}$
- *Coherence*: analogous to cross-correlation in the frequency domain between activity in two channels
- *Phase*: a measure of timing of activity between two channels
- *Symmetry*: between homologous pairs of electrodes

Measures of *absolute* or *relative* power can be derived from spectral analyses. Whereas absolute power reflects the amount of a given frequency within the EEG, relative power is calculated as the amount of EEG activity in a given frequency band divided by the total power. In general, absolute power should be preferred because it can be more easily interpreted.

By providing quantitative data regarding the changes in these bandwidths, QEEG could give us crucial information about several physiological and pathological conditions such as: cognitive decline, neuropathic pain, ADHA, dementia, learning disabilities and traumatic brain injury to mention just some, QEEG is highly reliable and reproducible technique that can offer a different perspective to the clinicians, especially when considered the complementary role of QEEG with other imaging/neurophysiological studies (Collura, 2010).

Below we can observe a typical EEG transformation by applying quantitative methods, from the raw signal to the spectral analysis and topographical representation based in the 10/20 international system, some neurological and psychiatric condition can demonstrate specific patterns, these disease oriented characteristics can help clinicians to guide treatments, or for research as an assessment tool in the methodology design.

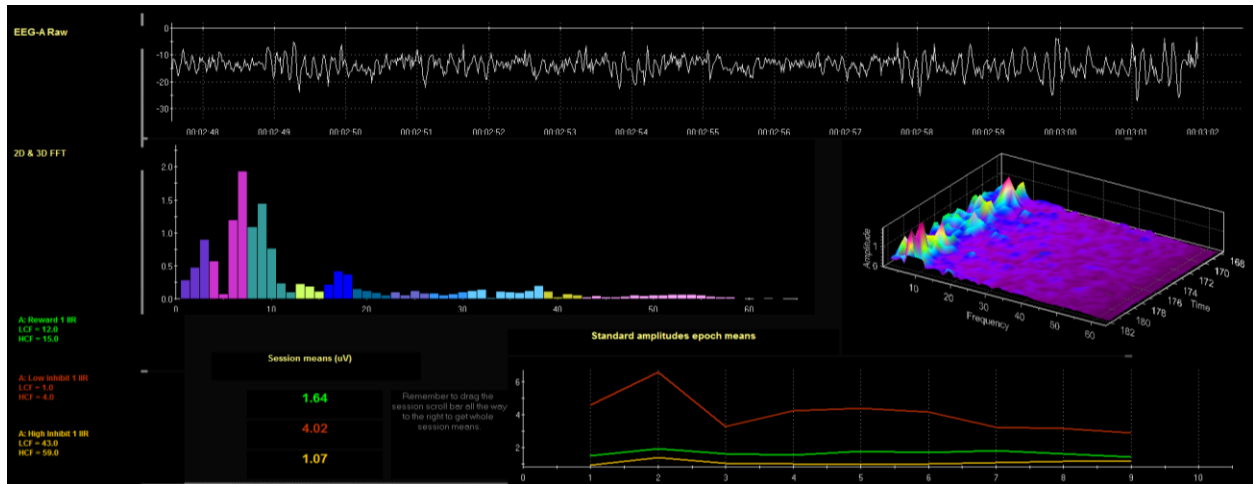


Figure 2. 8. Raw EEG signal and spectral analysis Top of the figure presents one channel of raw EEG signal with characteristic alpha oscillations (8-13 Hz), below the transformation of the spectral analysis where the 8 to 11 Hz power is being more prominent, bottom of the figure is the topographical analysis presenting increased power on the occipital areas. This EEG study was recorded under the eyes closed condition, therefore is characteristic of a normal adult EEG state.

Consistently with the main objectives of this research, QEEG can be considered sensitive and specific to detect changes in cortical electrical activity, that to be correlated with cognitive processes and performance, moreover, QEEG is the ideal tool to capture neurophysiological measurements of sustained electrical stimulation especially because the temporal resolution allows the observation of such changes immediately after the exposure to the intervention.

2.6 Relevant Functional Neuroanatomy

Early in the introduction of this manuscript a relevant concept was introduced, based in a previous research done by Datta, et al, a computer simulation of specific tPCS montage provide an insight about how a bi-auricular electrode montage might affect different cortical structures (A. Datta, J. P. Dmochowski, B. Guleyupoglu, M. Bikson, & F. Fregni, 2013), within this model a representation of current behavior showed direct influence over temporal and prefrontal areas (figure 2.9)

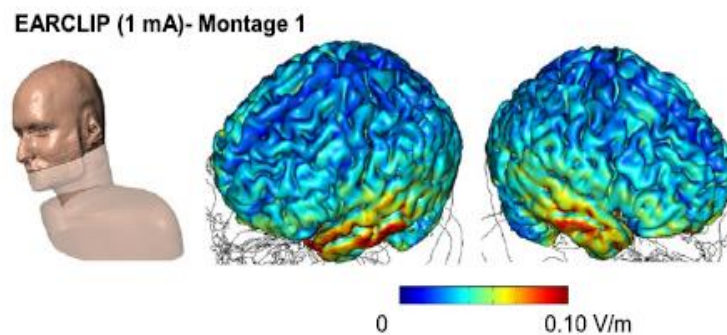


Figure 2. 9. Computational modeling of tPCS. High resolution computational current modeling of tPCS, based on this simulation the cortical areas influenced by tPCS are mainly located in the temporal and frontal structures. Adapted from Datta, et al. 2013.

The Frontal lobes are considered main structures for the function of cognition, social behavior and personality. Anatomically, the frontal lobes are located in the area found anterior to the central sulcus and above the lateral sulcus. The frontal lobes are complex structures as highly sophisticated functions are integrated within its boundaries. The frontal lobes are responsible for the capability to participate in the processing of abstract thought, it also facilitates planning and

the organization of behavior, and provides inhibition of inappropriate emotional and social reactions. Some of the functions that can be attributed to the frontal lobes are listed below;

- encoding and retrieval
- working memory
- attention and reasoning
- planning and executive functions
- motor movement and preparation
- Intelligence and reasoning

These are regarded as higher functions and for many authors represent the essence of the self and what differentiate humans from the rest of other species (Goldstein, 1990). The frontal lobes are also specialized per specific anatomical regions and the prefrontal cortex stands alone as a center containing the more advanced forms of thinking, also known as the executive functions, among them;

- divided and sustained attention
- speed processing and initiation
- sequencing and set-shifting
- Planning and cognitive flexibility

According to Luria, the prefrontal cortex (PFC) is essential for making goals, planning, and regulating behavior (A. R. Luria, Simernitskaya, & Tubylevich, 1970). Due its importance the frontal lobes occupy a large portion of the cerebral hemispheres as compromise about one-third of the cerebral cortex surface (Fischer, 1987). The frontal lobes are considered a main center for integration and association for the different areas of the frontal cortex and the limbic system. The

limbic connections may allow the frontal lobes, especially their paralimbic components, to link the sensorial aspects of external events with the visceral and emotional states they elicit (M. M. Mesulam & Geschwind, 1978). There are thirteen distinctive structural areas in the frontal lobes, and Brodmann distinguished them by their specific structure and functions (Brodmann, 1909), each of these neocortical areas present precise cytoarchitecture that makes them functionally specific, together, they create bigger lobar structures that are recognizable thanks to their anatomical localization. Thus, the premotor area (PMA), PFC, and the precentral area form the motor cortex. The more anterior aspect of the frontal cortex contains the premotor region and the supplementary motor area, the orbitofrontal cortex, and also Broca's area. The more anterior portion or frontal pole is the prefrontal region which is involved in the processing of execution of abstract thoughts, it also modulates inhibition of responses and reasoning, the dorsolateral cortex which controls working memory, and attention, and the orbitofrontal area are involved in acquisition, association, self-regulation and complex decision making.

Neuropsychological test results in patients with frontal lobe dysfunction are consistent with the critical role of this area in several cognitive domains such as; working memory, executive function, and the inhibition of inappropriate impulses. Thus, concentration (as assessed by digit span), the ability to resist interference, hypothesis testing (as assessed by the *Wisconsin Card Sorting Test*), the ability to maintain a coherent stream of thought, the ability to scan mental content (as assessed by verbal fluency and memory retrieval tasks), the ability to resist immediate but inappropriate response tendencies (assessed by the go-no go task), and the ability to internally program, select and sequence responses are usually impaired after prefrontal lesions (M. Mesulam, 2008). The connectivity pattern among the frontal lobe and deeper and distant neural structures demonstrates its role as an associative center (figure 2.10), the limbic

connections through the paralimbic nuclei serve as integrator of sensory information including those from visceral and emotional states. This connectivity pattern can be used as a shortcut for NIBS for autonomic neuromodulation, the influence in visceral activity associated with cognitive performance can be controlled under special circumstances, in the research setting, by applying NIBS while performing some specific task, this visceral modulation might facilitate some degree of stress response in an indirect way. It has been reported that damage to the orbitofrontal or medial components of the frontal lobes can interfere with the interactions between behavior and visceral state and may provide a physiological substrate for poor judgment and foresight (Damasio, 1995).

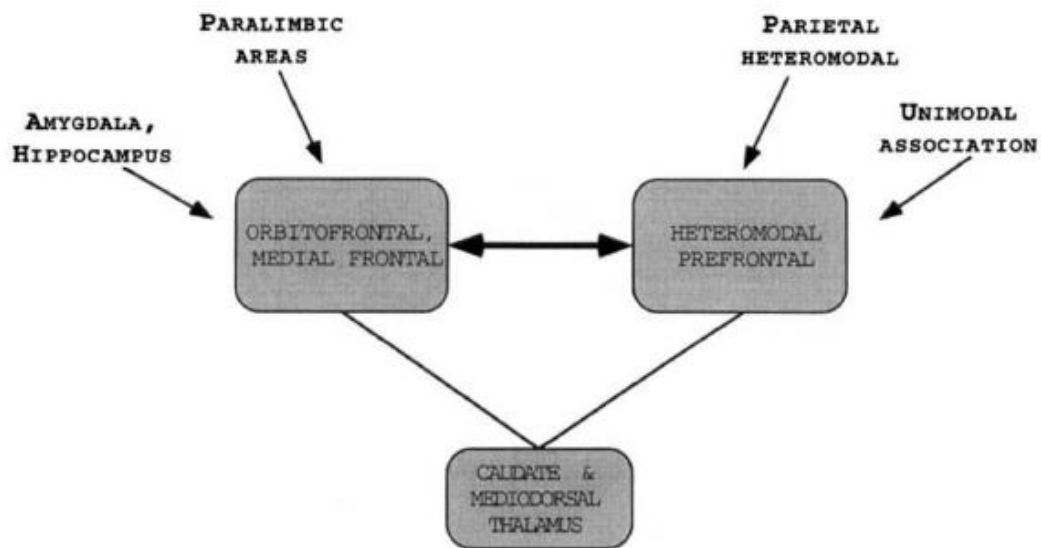


Figure 2. 10. Prefrontal cortex, major connections of the prefrontal cortex, from Mesulam, 2000.

The *metaphysiology* of the prefrontal cortex is characterized by its heterogeneity in regards structure, physiology, and connectivity patterns; this is what makes the PFC an area with multiple set of behaviors and cognitive functions which highlights its specialized organization, there are six distinctive attributes of PFC methaphysiology based in Mesulam theoretical model:

1. Even in the presence of significant damage to the PFC leaves sensation, movement, perception and homeostatic functions intact.
2. PFC has a high degree of interconnection density within heteromodal and unimodal neural centers, including limbic and paralimbic regions of the neocortex.
3. PFC shows preferential activity during response inhibition tasks, demonstrating its importance in inhibiting impulses for an adequate cognitive and behavioral performance.
4. Most PFC neurons that respond to visual stimuli present better discrimination for behavioral relevance than for visual characteristics, this phenomena the specialization of PFC neurons to coding and anticipation of behavior rather than just visual processing.
5. The functionality of PFC in working memory suggest a strong functional relationship with other neural structures involved in memory processing, the connectivity between PFC and temporal centers are considered highly interactive upon information storage and processing.
6. The orbitofrontal cortex and its connectivity with paralimbic structures provide the association for transmodal binding of thoughts, memories, and experiences with emotional and visceral states, this relationship facilitates the ability to cope and guide behavior during complex or ambiguous situations.

Despite all the advances in medical and cognitive research, still difficult to understand the complexity and uniqueness of the frontal lobe, thanks to the developments in brain imaging, genetic profiling and neurophysiological assessments we can elucidate some of the functions associated with frontal lobe processing, however, still area to cover when thinking of the frontal

lobe as target for therapeutic interventions, specially, when accurate definition of deficits are challenging to described even in the presence of massive damage to the frontal pole.

The temporal lobe it is mainly recognized as the cortex responsible for audition, language comprehension, memory, and learning. Although its role in audition is of great relevance, the focus of this review will explore the temporal lobe role as a heteromodal center for memory and learning. As mentioned before, the main current simulation model it also showed a broad activation of the temporal lobe (figure 2.9). Memory and learning are two of the most challenging functions of the human CNS, memory and learning are highly sophisticated processes that involve many components and structures within the temporal lobe but also outside of it. Most of the research done in memory distinguishes two different components: declarative memory which refers to the recall and information recognition of facts or events that are accessible in the form of conscious recollection; procedural memory is the memory process involving skills for automatic performance so it has some components of motor learned function for physical activities. For declarative memory the recollection of material previously learned and retrieved represents the conscious learning through memorization. For implicit memory the material recall is not retrieve in a deliberate fashion but rather occurs incidentally.

The main anatomical substrates for declarative anatomy are the hippocampus, PFC, and amygdala. The hippocampus and the parahippocampal region are the structures heavily interconnected for the process of declarative memory; these two structures form a system for the resolution of neocortical memory representations. The subdivisions of the parahippocampal region are interconnected and send principal efferents to many subdivisions of the hippocampus itself (green), the dentate gyrus, the CA3 and CA1 areas, and the subiculum. So the parahippocampal region serves as a convergence site for cortical input and mediates the

distribution of cortical afferents to the hippocampus (Eichenbaum, 2000). The interconnectivity with the amygdala mediates the processing of “emotional memories” while perceptual information as well as information about behavior is processed in many dedicated neocortical areas. This processing includes complex cognitive rules and concepts, such as those likely to be processed in the prefrontal cortex (Fuster, Bodner, & Kroger, 2000) .

Contrary to declarative memory, it seems semantic non procedural memory does not relay in the middle temporal lobe and in contrast with declarative memory, semantic memory neural substrates are not as well defined. The entorhinal cortex (ERC) and the hippocampal regions are the structures presenting higher activation during task involving semantic memory when traced on fMRI.

The perirhinal cortex (PRC) is located in the anteromedial temporal lobe, corresponding to Brodmann areas (BA) 35 and 36. Its medial component, it borders the ERC (BA 28) and anterolaterally, the temporopolar cortex (TPC). Although traditionally referred to as BA 38, the ventromedial aspect of the temporal pole has come to be regarded as an extension of BA 36 (Suzuki, Tsukiura, Matsue, Yamadori, & Fujii, 2005), and hence part of the ‘total perirhinal cortex’(Munoz & Insausti, 2005). Still quite controversial the exact group of neocortical structures involved in semantic memory, although this revision is not intended to fully cover the nature of semantic memory, it helps to understand that procedures requiring a rather available knowledge beforehand, demand an extensive activation through the semantic memory network.

The complexity of memory and learning are fully dependent on those mechanisms behind plasticity, the traditional Hebbian model where LTP/LTD helps to frame the electrochemical activity occurring in the neuroanatomy containing these neural structures, table 2.1 summarize

the intricate neural basis of memory and learning by activation using positron emitting tomography scanning (PET scan) and fMRI, it just gives a glance of how memory and learning interact in a rather intricate group of networks and systems (Cabeza & Nyberg, 2000).

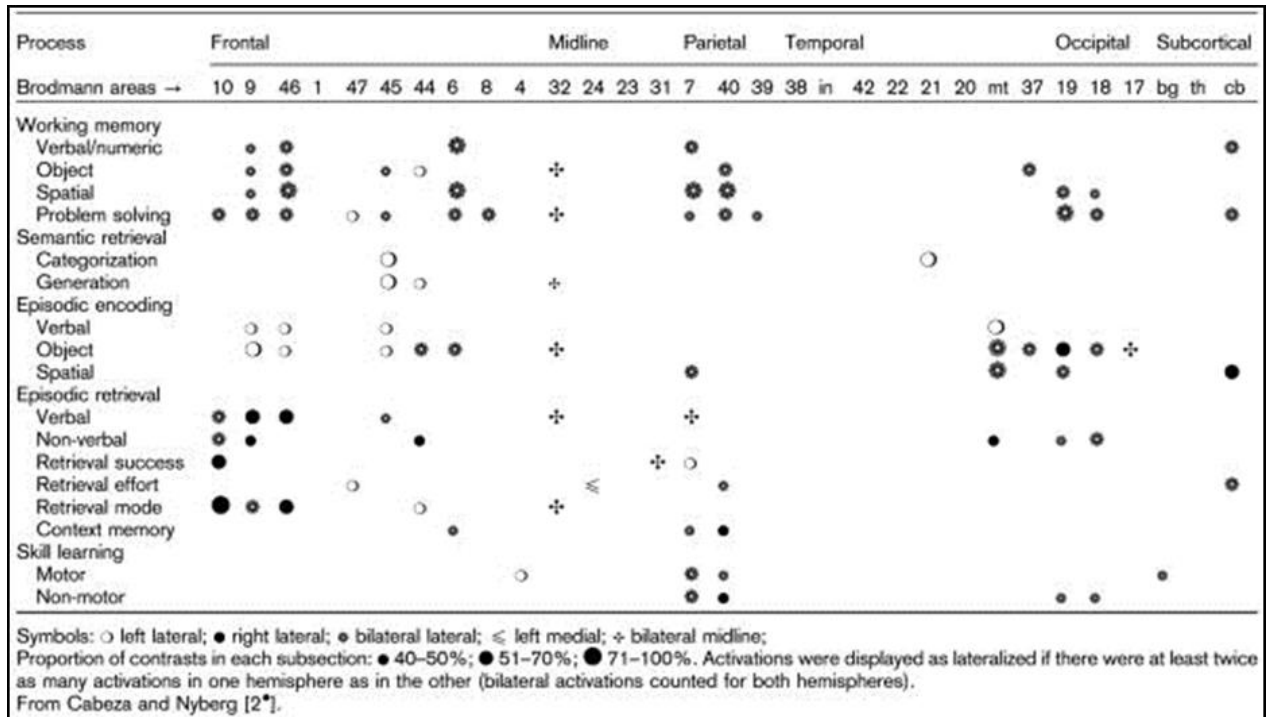


Table 2. 1. Neural structures demonstrating typical positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) activations during memory tests. Note the relevance of the frontal cortex for different components of working and episodic memory. From Cabeza & Nyberg, 2000

2.7 Transcranial Direct Current Stimulation and Transcranial Magnetic Stimulation as a model of noninvasive brain stimulation

There is very few literature in regards tPCS, although the field recognizes CES and tACS, tPCS still not very well define partially because the majority of the people applying the technique are clinicians who do not pay much attention about the electrical differences and mechanisms of action, also, CES has been already approved by the FDA and the term is currently being used as an “umbrella” for many other alternated current techniques. Therefore, a comparison we the two

most used neuromodulation tools is presented here, as to better understand the differences and the properties own by tPCS.

Transcranial direct current stimulation (tDCS) is a noninvasive method of neuromodulation where a weak direct current (DC) of 1-2 mA is applied via anodal and cathodal scalp electrodes that penetrates the skull and enters the brain. Although there is significant shunting of current in the scalp, adequate current penetrates the brain to modify transmembrane neuronal potential (Miranda, Lomarev, & Hallett, 2006) (Wagner et al., 2007), thereby influencing the level of excitability and modulating the firing rate of individual neurons. A series of tDCS experiments on the cortex of rats and cats in the 1960s demonstrated that weak anodal polarization increases cortical excitability and the firing rate of tonically discharging neurons, whereas cathodal polarization decreases excitability and firing rates.

When tDCS is applied for several minutes, cortical function may be altered for minutes to hours beyond the stimulation period, depending on the duration and strength of the polarization (Nitsche & Paulus, 2001). Excitability shifts during tDCS are believed to be due to subthreshold neuronal membrane depolarization, presumably caused by alterations in transmembrane proteins, ionic changes, and electrolysis-related changes (Nitsche, Nitsche, et al., 2003).

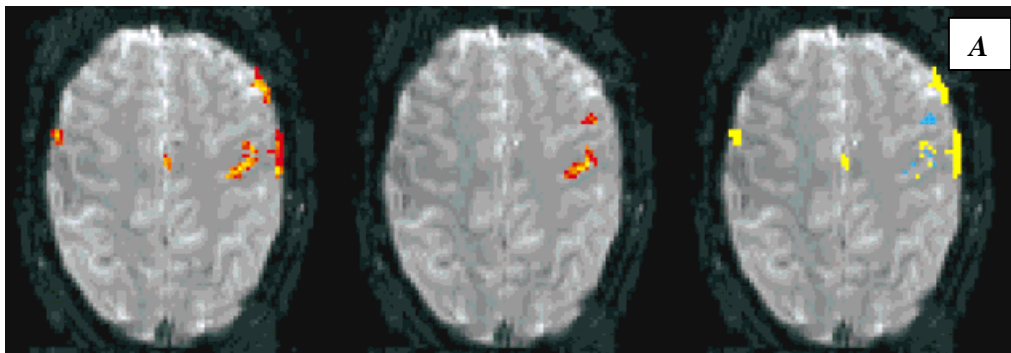
Studies have shown that tDCS can alter memory function in humans. For example, Dr. Fregni and colleagues found that applying anodal tDCS to the left DLPFC in neurotypical adults resulted in improved working memory performance (Fregni et al., 2005). Using tDCS to the anterior temporal lobes, Chi, Fregni, and Snyder (2010) found that applying left cathodal stimulation and right anodal stimulation resulted in enhanced visual memory function in neurotypical adults. Boggio also found that anodal tDCS to the left DLPFC enhanced working

memory performance in individuals with Parkinson's disease and that anodal tDCS to left temporal cortex enhanced visual recognition memory function in individuals with Alzheimer's disease (Boggio et al., 2009).

Electroencephalography (EEG) is used to measure electrical activity of the brain, by attaching electrodes to the scalp. EEG is often used as an adjuvant tool to control stimulation parameters of tDCS and to measure whether tDCS-induced behavioral changes are accompanied by changes in power amplitude, indicating enhanced neural processing. Although tDCS has most of its neuromodulatory effects on the underlying cortex, tDCS effects can also be observed in distant neural networks. Therefore, concomitant EEG monitoring of the effects of tDCS can provide valuable information on the mechanisms of tDCS. In addition, EEG findings can be used as important marker for the effects of tDCS and thus can be used to optimize its parameters. This combination of EEG-tDCS system can also be used for preventive treatment of neurological conditions characterized by abnormal peaks of cortical excitability, such as seizures (Schestatsky, Morales-Quezada, & Fregni, 2013). By using EEG to monitor tDCS affects Matsumoto observed an increase of the event-related-desynchronization (ERD) for the mu rhythm which oscillates between 8 to 13 Hz after anodal stimulation, whereas cathodal stimulation produce the opposite (Matsumoto et al., 2010). EEG has been also used to compare and correlate the effects on cortical excitability measured by motor evoked potentials (MEP), cathodal stimulation of the primary motor cortex decrease the MEP amplitude and increase the power spectrum of the delta and theta bands, these findings demonstrate that the after-effects of tDCS have a non-synaptic mechanism of action based upon changes in neural membrane function. These changes apart from reflecting local changes in ionic concentrations could arise from alterations in transmembrane proteins and from electrolysis-related changes induced by

exposure to a polarity specific constant electric field (Ardolino, Bossi, Barbieri, & Priori, 2005). The effects of tDCS have been captured on functional imaging as well. Blood oxygenation level dependent (BOLD) MRI was used to monitor the changes produced by tDCS on the sensorimotor cortex (figure 2.11). Cathodal stimulation resulted in a global decrease of the mean number of activated pixels 0–5 min after stimulation. A region-of-interest analysis revealed a 57% decrease of activated pixels in the supplementary motor area, but no change in the hand area of the primary motor cortex. In the other hand anodal stimulation yielded a nonsignificant increase of activated pixels with no regional differences. These findings support the view that reduced neuroaxonal excitability after cathodal tDCS causes reduced brain activity (Baudewig, Nitsche, Paulus, & Frahm, 2001).

Following tDCS safety guidelines (Nitsche, Liebetanz, et al., 2003) and recognizing most of the reported side effects (Poreisz, Boros, Antal, & Paulus, 2007), tDCS can be considered as a safe instrument to induce neuromodulatory cortical changes to investigate several cortical functions, the main question to answer is if tPCS has the same safety profile.



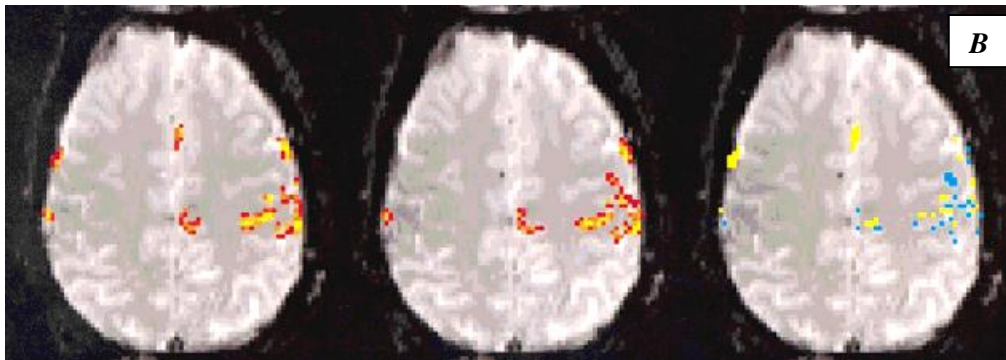


Figure 2. 11. BOLD activation maps depicting one subject exposed to: A cathodal stimulation, B anodal stimulation. Left panel shows activation after finger tapping task, middle panel after 5 minutes of stimulation, right panel is the “binary difference” from pre to post stimulation. Notice the decrease in the activation maps for cathodal while anodal produce the opposite effect. Adapted from Baudewig., *et al.*,2001.

Transcranial Magnetic Stimulation (TMS) is a relatively new brain stimulation modality. Rather than modulate at subthreshold level as in the case of tDCS, TMS truly induces neuronal action potentials, TMS noninvasively stimulates an area of the brain, and when evoking a response, establishes a causal relation between brain activation and behavior.

The TMS device is magnetic stimulator consisting of a bank of capacitors connected to a wire coil. When the electrical energy stored in the capacitor is released into the coil, a large current of several thousand amps with rapid rise time of about 200ms flows in the coil and then decay back to zero in about 1ms. According to Faraday’s Law, the time-varying current flowing in the coil induces a magnetic field (Baker, 1985). The transient magnetic field causes electrical current to flow in excitable tissues, such as neurons or axons in the brain, and the magnetic field strength falls off rapidly with the distance from the coil (Hess, Mills, & Murray, 1987).

As a new therapeutic alternative, repetitive Transcranial Magnetic Stimulation (rTMS), a noninvasive brain stimulation technique, have been proposed. The technique is based on the electromagnetic induction principle, which is capable of eliciting neuronal depolarization with use of coils placed over patients’ scalp. When this depolarization occurs in a repetitive way,

cortical modulation may persist even after the stimulus is removed. The cortical facilitation or inhibition reached depends on the frequency and intensity of pulses repetitions (Hummel & Cohen, 2006).

TMS when given in form of pulse trains (rTMS) can be used to modulate cortical activity by either up-regulating (excitatory effect) or down-regulating (inhibitory effect) cortical excitability depending on rTMS parameters used. The motor cortices in the left and right hemispheres of human brain are strongly interconnected, with each side naturally inhibiting the activity of the other side – and achieving a natural balance. If one side is lesioned as in stroke, however, its activation is decreased and its inhibition to the other side is reduced, leading to increased activation in the non-lesioned side. Additionally, the non-lesioned side still provides inhibitory signals to the lesioned side, even more than in the prior healthy balance situation. This mismatch leads to a condition where the lesioned hemisphere cannot easily deliver action potentials to the lower motor neuron and the corresponding muscles. As result, the ability to participate in motor training which is necessary for recovery of function is severely challenged. Besides motor or language functions, TMS has been used to explore cortical areas involved in cognitive processing. The modulation of higher mental functions has been gone in two directions; 1) inhibiting certain cortical arear and producing what has been called “virtual lesion” or 2) by enhancing the excitability of a specific cortex involved in cognition so “facilitation” of function can be observed.

In a study on cognitive functioning rTMS was used to explore and evaluate the role of DLPFC in memory-guided responses for two different types of spatial working memory tasks. A 10-Hz train of TMS was delivered at the onset of the response period. Researchers found that only DLPFC rTMS significantly affected performance, with rTMS of right DLPFC decreasing

accuracy on delayed-recall trials, and rTMS of left and right DLPFC decreasing and enhancing accuracy, respectively, on delayed-recognition trials. These findings confirm that the DLPFC plays an important role in memory-guided response, it was also observed the differential effects of rTMS had on enhancing or inhibiting memory processing depending of coil localization (Hamidi, Tononi, & Postle, 2009). The potential for rTMS to somewhat replicate the effects of cortical lesions, has been proven by pre selecting integration areas and exposed them to inhibitory rTMS. Spatial attention was impaired when rTMS was delivered to the ipsilateral parietal cortex, mimicking the symptoms observed in patients with hemi-neglect syndrome (Hilgetag, Theoret, & Pascual-Leone, 2001). In the opposite side of this, rTMS can also modulate positively the performance of cognition by applying different parameters of stimulation. By selecting a pre-defined upper level for the alpha EEG band (8-13Hz) + 1Hz of TMS (e.g. 10 EEG alpha HZ + 1 TMS = 11 rTMS Hz for stimulation) demonstrated increased modulation in the power of the alpha band, but also an increase in performance for a mental rotation task when the mesial frontal and parietal cortex were stimulated (Klimesch, Sauseng, & Gerloff, 2003), the relevance of this study is that based on QEEG rTMS showed potential to entrain electrical cortical oscillation and this was associated with improved performance in a specific cognitive task. Altogether, we can assume TMS have a direct effect in neuronal depolarization which in turn facilitates the release of neurotransmitters, if the frequency is excitatory (Kikuchi et al.) will induce glutamatergic release, while at low frequencies (<5Hz) an inhibitory effect will be associated with glutamatergic release. Considering these mechanisms and understanding its role for neuroplasticity, both tDCS and TMS are ideal tools to be used in neurology, psychiatry, psychology, and rehabilitation. However the same question still elusive if

tPCS is added to the equation, while tDCS seems to act at subthreshold membrane level and TMS drives the neuron to actually depolarize due to the intensity of the generated electrical field

3. METHODOLOGY

These investigations were conducted in a single research facility, double-blinded, sham-controlled, randomized trial at the Neuromodulation Center, Spaulding Rehabilitation Hospital, with the objective to explore the effects of a single-session of tPCS vs. Sham stimulation on cortical activity and performance on cognitive and behavioral tasks. To determine the mechanistic properties of tPCS, four experiments were designed with the purpose to test different parameters of stimulation and its effects on electrical cortical activity, behavioral and cognitive functioning. A table with the studies characteristics and a flow diagram are presented below.

These studies were approved by the Spaulding Rehabilitation Hospital ethics review committee and De Montfort University (Leicester, UK) ethics review committee. All participants enrolled provided written informed consent. A total of 140 participants were selected to take part in this study. All enrolled participants met the following inclusion criteria for the present study included; 1) Healthy subjects aged between 18 and 65 years old. Exclusion criteria from the present studies included; 1) existence of major neurologic or psychiatric condition (i.e. epilepsy, severe depression), 2) history of head injury resulting in more than momentary loss of consciousness, 3) previous neurosurgery, 4) history of alcohol or drug abuse, 5) presence of unstable medical condition such as; diabetes mellitus, cardiac pathology, cancer, kidney insufficiency, 6) contraindications for tPCS, such as; metal in the head, implanted electronic medical devices and 7) female participants were excluded if a urinary pregnancy test came positive, participants voluntarily accepted the evaluation and the testing was performed in the

privacy of a restroom stall, the testing procedure included the presence of a female member of the research team to assure reliability of the testing as the participant handled the test result to the team member for its evaluation.

Time Period	Assessment	1st Experiment n=40	2nd Experiment n=40	3th Experiment n=30	4th Experiment n=30
	<i>Consent and Screening</i>	X	X	X	X
Before and After Stimulation	<i>qEEG</i>	X	X		
	<i>MMSE</i>	X	X	X	X
	<i>Stroop test</i>	X	X	X	X
	<i>WLMT</i>	X	X		
	<i>BART</i>			X	
	<i>Arithmetic task</i>			X	
	<i>Paired-Associative learning task</i>				X
	<i>Attention switching task</i>				X
Pre and Post Stimulation	<i>HRV</i>			X	X
	<i>EDR</i>			X	X
After Stimulation	<i>tPCS side effects questionnaire</i>	X	X	X	X
	<i>Validation of Blinding</i>	X	X	X	X
<i>Approximate time</i>		<i>120 minutes</i>	<i>120 minutes</i>	<i>90 minutes</i>	<i>90 minutes</i>

Table 3. 1. Experiments and assessments schematic representation Abbreviations *qEEG*: quantitative EEG; *MMSE*: mini-mental state examination; *WLMT*: word learned memory test; *BART*; balloon analogue risk task; *HRV*: heart rate variability; *EDR*: electro-dermal response; *tPCS*: transcranial pulsed current stimulation.

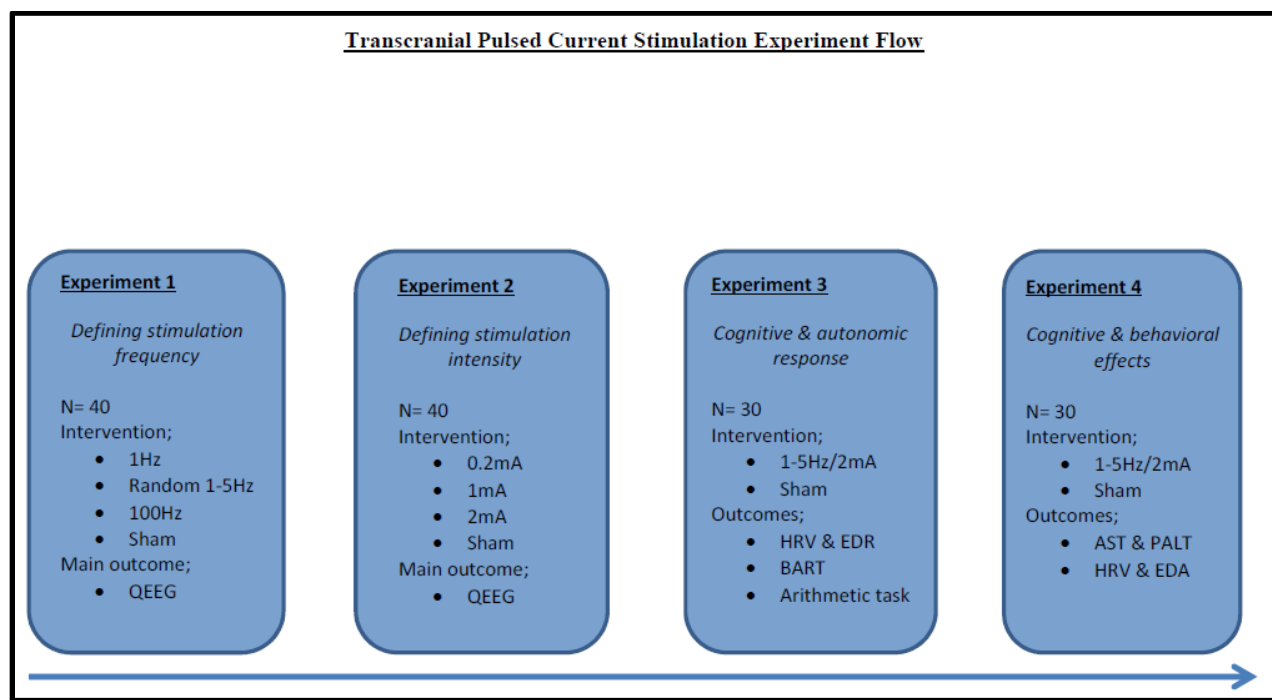


Figure 3. 1. Study flow showing the properties for each experiment. Experiments 1 and 2 were designed with the intention to explore the optimal parameters to observe cortical modulation as measured by qEEG. Experiments 3 and 4 used the parameters obtained from the previous two experiments (tPCS with a random frequency between 1-5Hz, current intensity of 2mA for 20 minutes), and applying such parameters to test its effects in cognitive and behavioral tasks. Abbreviations *qEEG*: quantitative EEG; *HRV*: heart rate variability; *EDR*: electro-dermal response; *BART*: balloon analogue risk task; *AST*: attention switching task; *PALT*: paired associative learning task *tPCS*: transcranial pulsed current stimulation

3.1 Experiment 1 (defining stimulation frequency)

Participants

Forty healthy volunteers were recruited to participate in this study; participants came from the Boston metropolitan area by posting ads in universities, colleges, public areas, and internet. Participants were eligible using the following inclusion criteria: age between 18 and 65 years; (2) no diagnosis of neurological, psychiatric, or metabolic disorder, as reviewed by a scientist physician; (3) no personal history of stroke, traumatic brain injury or epilepsy as reviewed by a scientist physician; (4) no drug or alcohol abuse; (5) no history of brain surgery or presence of metallic implants on the head; (6) pregnancy. All female participants were tested for

gravidity using a urinary pregnancy test. A female researcher accompanied and performed the testing in the privacy of a restroom stall. All participants completed a Mini Mental State Examination (MMSE) as a brief screening tool to assess and determine neurocognitive capabilities, this with the purpose to rule out the presence of deficits or any other sign of deviant performance that could be indicative of a dementia problem. The MMSE is a well validated and standardized neurological scale used in the clinical and research settings for a quick assessment of cognitive impairment overtime (Reisberg, et al., 1982), although the MMSE is not used as primary outcome its main purpose was to be used as an objective measurement of safety. Each participant received \$50 US dollars as monetary compensation for participation.

Out of the 40 participants that were recruited, 38 completed the single stimulation session. Two subjects signed the consent form and decided not to participate before stimulation was given. Nine participants (23.68%) were randomized to the sham group, 9 (23.68%) to 1 Hz stimulation, 9 (23.68%) to 100 Hz stimulation, and 11 (28.95%) to 1-5 Hz random frequency stimulation. One subject in the 1-5 Hz random frequency group was removed from the analysis due to excessive artifacts in the EEG recording. There were no significant differences in demographic characteristics between groups, table 3.2 represents sample characteristics.

	1Hz	Random 1-5Hz	100Hz	Sham
Male/female	3/6	6/5	6/3	4/5
Age, years	29.11 (13.21)	27.36 (9.37)	27.44 (8.58)	36 (12.36)

Table 3. 2. Demographic data at baseline. Mean (SD)

Design

This experiment was a double-blinded, sham controlled, randomized trial conducted at the Neuromodulation Center. The experiment contained 4 arms, which were run in parallel between-subjects design. Participants were randomized into one of four study arms: sham stimulation, (2) active stimulation with a frequency of 1 Hz, (3) active stimulation with a random frequency ranging between 1 and 5 Hz and (4) active stimulation with 100 Hz. beginning with Experiment 1 and ending with Experiment 4. Each participant was allocated to one of the intervention groups and having one visit that included baseline recordings and assessments followed by exposure to intervention, and finishing with post intervention measurements. The independent variable for each arm was the stimulation frequency at which participant were allocated, the dependent variable were the results obtained as a main outcome (qEEG), table 1 presents all of the dependent variables in the column named “assessments”, considering “time” as a factor each variable had two levels which were used for analysis; pre-stimulation and post-stimulation.

Materials

Transcranial Pulsed Current Stimulation (tPCS)

All participants were randomized into one of four study arms sham stimulation or active stimulation. To create the randomization list a computer algorithm was used to generate permuted blocks of four (www.randomization.com). In all of the study arms, both the investigator assessing the outcomes and the participants were blind to the intervention. The tPCS device used was an investigational, custom-made, and battery powered, high-frequency cycling stimulator, developed by BrainGear (BrainGear AG, Switzerland). This device delivers a pulsed,

low-amplitude electrical current, which is considered a specific type of transcranial alternating current with a biphasic temporal wavelength. Since tPCS generates a pulsed alternating bidirectional current, it does not matter where the anode or cathode are positioned following a bilateral pattern (earlobes). The associated pulse frequency and its relation to the magnitude spectrum randomly oscillate between 0 and .637A. The tPCS device was specially designed for these investigational applications. The tPCS unit has a user friendly interface and the person operating the system received training and was certified by the research coordinator (Figure 3.2).

For all of the experiments earlobe-clip electrode montage was used, saline soaked felt tissue covered the metallic electrode and prevented direct contact with the skin. Before the device was used for experimentation a non-blind research staff tested the devices for a measurement of quality assurance using a portable oscilloscope (DSO Nano, Seed Studio) (Figure 3.3 A and B) and a custom made LED plate pulse detector, as up to four devices were pre-programed to deliver the specific frequency and intensity (experiments 1 and 2) that the subject was randomized for.

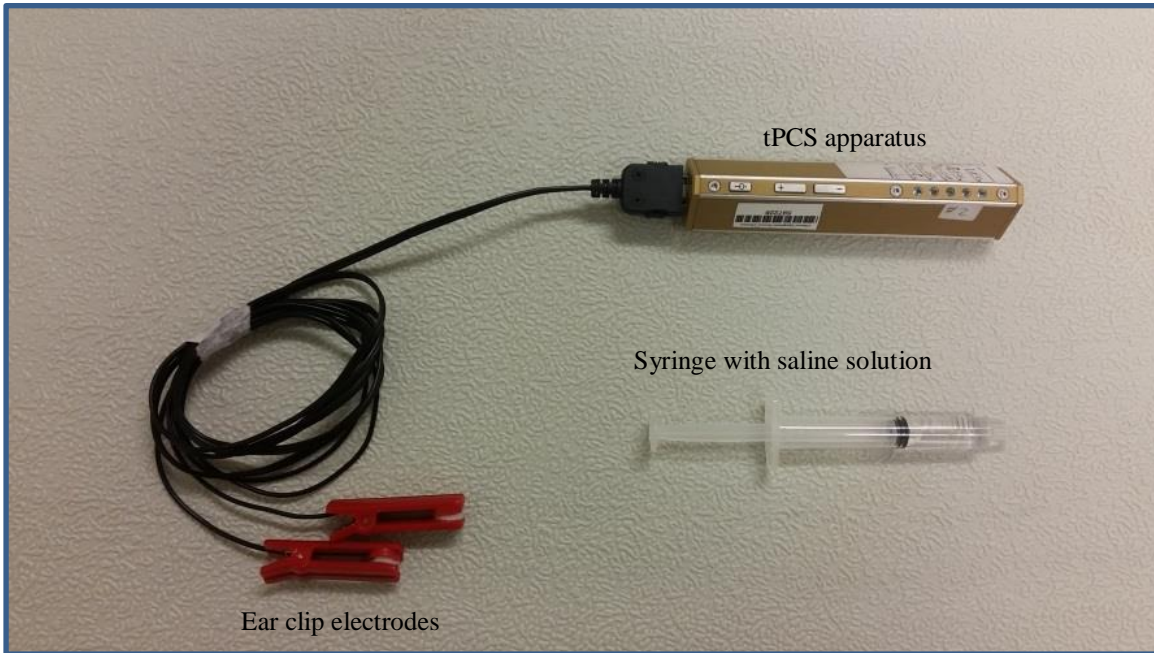


Figure 3. 2. Transcranial pulsed current stimulation (tPCS) apparatus with earlobe clip electrodes and saline solution

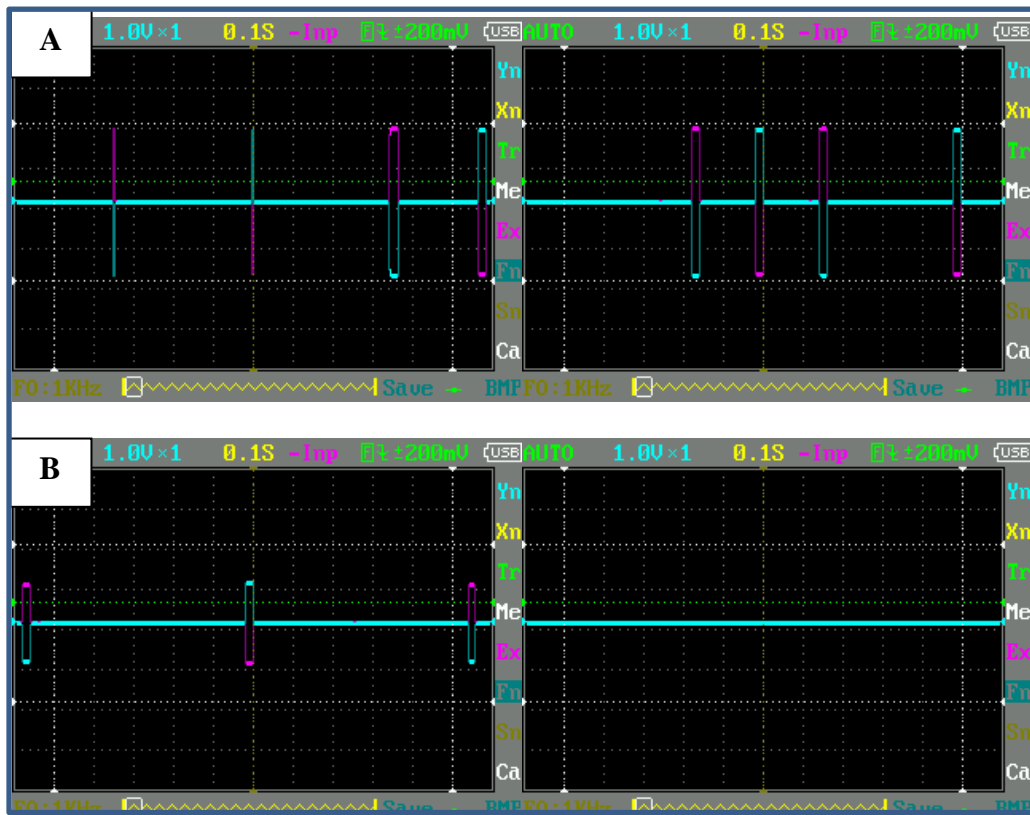


Figure 3. 3. Pulse frequency registration captured by oscilloscope registration, a 1kOhm resistor simulated skin impedance (typically 500-5k), so the 2mA current induced a 2V amplitude which appeared as +2V and -2V in the oscilloscope for different polarities, time interval is 1 second, traces showed two time point at 1-5Hz (A); and sham condition (B), which stimulated for 15 seconds at 1Hz followed by off stimulation).

Quantitative electroencephalography (qEEG)

Electroencephalographic recordings for experiment 1 were recorded using a high density array EEG (EGI, Oregon, USA), a geodesic sensor net with 64 channels was used for signal acquisition. Data were sampled at a rate of 256 Hz, amplified and filtered using a band pass analog-digital of 0.5-40 Hz. Power spectrum, band power, and intraband mean and median analysis for the EEG frequency bands were calculated by decomposing the raw signal being generated in different areas of the brain. The Fast Fourier Transformation (FFT) analysis was used for signal processing and spectral analysis to determine and measure the amplitude of the predominant EEG frequency, the resulting power spectra had 1Hz frequency resolution, representing frequency domain spectral amplitudes as a result of the Fourier coefficients, the average amplitudes were normalized with a 1 μ V peak amplitude in the time domain would result in a 1 μ V spectral amplitude in the frequency domain, the obtained properties in both time and frequency domains were used for its statistical analysis.

Coherence analysis was used to measure the coupling of large scale networks. Coherence is a frequency-dependent measure, mathematically obtained by dividing the cross-spectrum between two time series by the root of the two spectra (this computation is similar to a correlation; Cross-power spectrum is obtained by multiplying the Fourier transform of one signal with the complex conjugate of another signal, thus allowing the quantification of relationships between different EEG signals (Schack, Vath, Petsche, Geissler, & Moller, 2002).

Mini Mental State examination (MMSE)

The MMSE is a brief screening instrument used to assess cognitive abilities, specifically memory, the examination includes 12 questions and requires about 5 to 10 minutes to complete

the examination, and thus it is considered a useful tool easy to administer in the research settings (Folstein, Folstein, & McHugh, 1975). It has been used to assess patients with dementia symptoms as in the case of Alzheimer's disease, vascular related dementia, or age related dementia. The MMSE construct allows exploring some other cognitive domains such as; attention, recall, language, orientation and the ability to follow simple commands. The MMSE is the most widely used of cognitive screening tools. An examination of the psychometric properties of the MMSE seems warranted because the accurate and comprehensive assessment of mental status (Pangman, Sloan, & Guse, 2000). The assessment was used as a baseline evaluation and a follow-up, with the purpose to monitor cognitive capabilities on the tested subjects. Consistency of MMSE scores should suggest that a subject had no cognitive changes throughout the intervention period that may have affected test performance or carryover of the stimulation. The MMSE was used as a measurement of safety as no other research using tPCS evaluated its immediate effects on higher mental functions before.

Neuropsychological assessments

Stroop test

This test is based on the Stroop Effect found by John Ridley Stroop in 1930's (Stroop, 1935). In the Stroop test the subject is presented with names of colors written in the same color or in a different color, thus on the one hand the word names a color (red) and is written in another color (Parsons et al.). It has been found that naming colors of words takes longer than reading color names. This is called the Stroop Effect. In the Stroop Task the automatized behavior (Reading) is in conflict with the desired response (naming the color). The Subject has to inhibit/suppress the automatic response of reading and name the color the word is written in. The

Stroop test is one of the most commonly used tools for determining attentional problems. It is also a test of Executive Function and Working Memory. Executive functions are intrinsic to the ability to respond in an adaptive manner to novel situations and can be conceptualized as having four components: (1) volition; (2) planning; (3) purposive action; and (4) effective performance (Lezak, 2004), therefore, the Stroop test is a valid assessment of inhibition, fluid ability, and speed, these cognitive functions correspond to frontal lobe processes that can be evaluated with the Stroop test having an adequate test-retest reliability and ecological validity (Baddeley & Wilson, 1988).

Research on executive functions has its origins in the study of patients with frontal lobe lesions; it has been known that patients with frontal lobe damage exhibit poor regulation of their behavior (Aleksandr Romanovich Luria, 1966), hence, if tPCS has a detrimental acute effects on frontal/prefrontal lobe functions, then the Stroop test is a sensitive tool to monitor these changes overtime.

Word List Memory task (WLMT)

This is a memory task for assessing word list recall; it was used with the intention to evaluate the effects of tPCS on semantic memory. A semantic memory task where person's general knowledge of vocabulary is tested by presenting an auditory stimuli consisting of a set of words, it is a practical measurement of concepts recollection, being also sensitive to acute changes or memory fluctuations when an intervention is applied. In this experiment the WLMT was used as measurement of safety, as tPCS has been shown to affect temporal lobe structures by computer modeling (A. Datta, et al., 2013). Neural correlates of memory include; hippocampus, entorhinal cortex, temporal cortex and its connections with prefrontal structures (Woodruff,

Johnson, Uncapher, & Rugg, 2005), as these circuits can be affected by tPCS modulation, the WLMT offered the advantage to explore for immediate and delay recall and recognition of auditory material. The task was generated using custom made generic software created in www.google.com and the Paivo *et al.* (Friendly, M. 1996, Paivo *et al.* *Word List Generator*, <http://www.datavis.ca/online/paivio/>) word list generator environment and using a neutral voice to speak out the set of words presented through headphones. The volume of the word list was adjusted to assure good quality of the auditory signal; each participant selected the adequate volume for his/her audition. The task involves presenting the subject with a list of 10 high-frequency, high-imagery words. The generator presented a set of words which properties such as; number of syllables; number of letters; concreteness and meaningfulness ratings followed normal distribution in an extended inventory of words contained in general English lexicon. The words are read to the subject at a constant rate of 1 word every 2 seconds. The word list is presented 3 times to the subject; the order of words is randomized for each trial. At the end of each of the three presentations, the subject is asked to recall the list of words; all responses were registered after recall in an evaluation sheet, the written words after recall were compared with the presented WMLT previously presented to the participant, accurate responses and mistakes were the main outcomes for the assessment of this task.

tPCS side effects questionnaire

After the stimulation session, participants completed a questionnaire for tolerability of stimulation on a 5-point scale. The subjects were asked whether they have experienced any discomfort in an open-ended manner and they will then be specifically asked about headache, neck pain, scalp pain, scalp burns, tingling, skin redness, sleepiness, trouble concentrating, and

acute mood change. This is the same questionnaire used in all of noninvasive brain stimulation trials. If any side effects are reported, the degree of relatedness to the intervention will be assessed.

tPCS blinding questionnaire

After the stimulation session, participants completed a questionnaire to determine if our blinding methods were effective. We used a 30 seconds sham montage (15 second at the beginning and at the end of 20 minutes of supposed stimulation), just as we use in other trials involving NIBS, keeping the device on the subject for the duration of the session.

Study Procedures

Forty participants were enrolled for this experiment. Each participant went through the screening procedures as outlined above, and randomized to receive one of the 4 proposed tPCS frequencies: 1Hz, random freq, 1-5Hz range, 100Hz and sham (a total of 10 participants per frequency condition). After consent and screening were obtained, participants completed the following:

- MMSE
- The Stroop test
- Word list memory task
- 64 channel high density array electroencephalography (EEG) – was collected by using the traditional methodology of eyes open (EO) and eyes closed (EC) for 10 minutes each; Subjects were sited in a comfortable position, a technician placed the previously soaked

in potassium chloride solution the EEG geodesic net onto participant head. The recordings were done having the subjects in wake restful condition and seated in a comfortable chair. Subjects were instructed to keep his/her focus in a fixed point marked on the wall in front of them. The EEG technician directed each subject to avoid excess of head movement, eye blinking, eye rolling, muscle tension or any other motion capable to produce non EEG artifacts on the recordings. During the EEG recordings the environmental noise was kept at minimum and participants were asked to keep their thoughts as neutral as possible (i.e. avoiding thoughts about the monetary compensation or how much time was left into the experiment)

Stimulation: after initial assessments, participants underwent one session of tPCS, for 20 minutes at 2mA, at the frequency to which they have been previously randomized (1Hz, random 1-5Hz, 100Hz or sham). Previous to the stimulation a research explained the procedure and described the feeling of the stimulation. A saline soaked pelt sponge over the stimulation electrode was mounted in each participant's ear lobe with an ear-clip. The Stimulation was ramped up during the first 30 seconds of the procedure and after this period the current intensity reached its maximum parameter, by the end of the stimulation period the current was ramped down for the final 30 seconds. Participants in the sham group received only the ramping periods at the beginning and at the end of the 20 minutes of sham stimulation.

After stimulation participants completed the above mentioned assessments for post-stimulation evaluation. Additionally, participants also completed:

- tPCS side effects questionnaire
- Blinding questionnaire

Statistical Analysis

Analyses were performed using Stata Version 13 statistical software (StataCorp LP). To compare baseline characteristics between groups, we used a one-way analysis of variance for continuous variables and Fisher's exact test for categorical variables. To evaluate the effects of the intervention on interhemispheric coherence for each frequency band we used a mixed model analysis of variance using coherence difference between the post and pre-experimental conditionals the dependent variable, and the following independent variables: a within-subject variable for eyes condition (2 levels-open and closed), a between-subject variable of group (4 levels), and the interaction term eyes \times group. According to a-priori specifications, we used t-test for comparison to assess the differences between the random frequency group and sham, (2) 1 Hz and sham, (3) 100 Hz and sham. For power analysis, we used a similar model, using the differences between the post and pre-experimental condition of the mean power of alpha, theta and beta bands as the dependent variable. Post-hoc comparisons followed the same pattern explained above. For neuropsychological outcomes we used a one-way ANOVA using the difference between the post and pre-experimental condition between groups. We reported the frequency of adverse events in each group and used Fisher's exact test for assessing group differences. For validation of blinding, we used Fisher's exact test to assess differences between the four groups. For statistical significant results and in order to explore the magnitude of their effect, measures of effect size were calculated for ANOVA, and are presented as the correlation between an effect and the dependent variable. If the value of the measure of association is squared it can be interpreted as the proportion of variance in the dependent variable that is attributable to each effect. For the analysis of this experiment the eta squared (η^2) was used. The

calculation of effect size for paired t-test was performed using the mean difference between (mean control group – mean active group) groups and dividing over the standard deviation.

3.2 Experiment 2 (defining stimulation intensity)

Participants

Forty healthy volunteers were recruited to participate in this study; participants came from the Boston metropolitan area by posting ads in universities, colleges, public areas, and internet. Participants were eligible using the following inclusion criteria: age between 18 and 65 years; (2) no diagnosis of neurological, psychiatric, or metabolic disorder, as reviewed by a scientist physician; (3) no personal history of stroke, traumatic brain injury or epilepsy as reviewed by a scientist physician; (4) no drug or alcohol abuse; (5) no history of brain surgery or presence of metallic implants on the head; (6) pregnancy. All female participants were tested for gravidity using a urinary pregnancy test. A female researcher accompanied and performed the testing in the privacy of a restroom stall. All participants completed a Mini Mental State Examination (MMSE) as a brief screening tool to assess and determine neurocognitive capabilities, this with the purpose to rule out the presence of deficits or any other sign of deviant performance that could be indicative of a dementia problem. The MMSE is a well validated and standardized neurological scale used in the clinical and research settings for a quick assessment of cognitive impairment overtime (Reisberg, et al., 1982), although the MMSE is not used as primary outcome its main purpose was to be used as an objective measurement of safety. Each participant received \$50 US dollars as monetary compensation for participation.

Out of the 40 participants that were recruited, 39 completed the single stimulation session. One subject signed the consent form and decided not to participate before randomization was

performed. Ten participants (25.64%) were randomized to the sham group, 9 (23.07%) to the 0.2 mA intensity, 10 (25.64%) to 1 mA intensity, and 10 (25.64%) to 2 mA intensity. One subject in the 1 mA intensity group was removed from the analysis due to excessive artifacts in the EEG recording. There were no significant differences in demographic characteristics between groups, table 3.3 represents sample characteristics.

Demographics	0.2mA	1 mA	2mA	Sham
Male/female	4/5	5/5	4/6	4/6
Age, years	27.10 (3.85)	29.4 (3.92)	30 (4.83)	36 (12.36)

Table 3. 3. Demographic data at baseline. Mean (SD)

Design

This experiment was a double-blinded, sham controlled, randomized trial conducted at the Neuromodulation Center. The experiment contained 4 arms, which were run in parallel between-subjects design. Participants were randomized into one of four study arms: (1 sham stimulation, (2) active stimulation with a 0.2 mA, (3) active stimulation with intensity stimulation of 1 mA and (4) active stimulation with 2 mA. Each participant was allocated to one of the intervention groups and having one visit that included baseline recordings and assessments followed by exposure to intervention, and finishing with post intervention measurements. The independent variable for each arm was the stimulation intensity at which participant were allocated, the dependent variables were the results obtained as a main outcome (qEEG), table 1 presents all of the dependent variables in the column named “assessments”, considering “time” as a factor each variable had two levels which were used for analysis; pre-stimulation and post-stimulation.

Materials

Transcranial Current Pulsed Stimulation (tPCS)

All participants were randomized into one of four study arms. To create the randomization list a computer algorithm was used to generate permuted blocks of four (www.randomization.com). In all of the study arms, both the investigator assessing the outcomes and the participants were blind to the intervention. The tPCS device used was an investigational, custom-made, and battery powered. The same unit used for experiment one was used in this experiment, the only difference in the parameters of stimulation was the intensity frequency in which the unit delivered the current; 0.2 mA, 1 mA, 2 mA, or sham. The chosen frequency for stimulation was the 1-5 random frequency used in experiment one, time of stimulation was 20 minutes for all participants.

Quantitative electroencephalography (QEEG)

The EEG recording system used in this experiment was the same described above for experiment number 1. Methods for EEG acquisition followed the same parameters described previously.

Mini Mental State examination (MMSE)

As in the case of experiment number one the MMSE was used as measurement of safety.

Neuropsychological Assessments

For this experiment the Stroop test and the WLMT were applied with the same purpose as experiment one, both tests were used as measurements of safety.

tPCS side effects questionnaire and tPCS blinding questionnaire

Both questionnaires were used to evaluate the presence of any adverse effect related with the intervention and to validate the blinding method. The questionnaires were applied following the same approach described above.

Study Procedures

Forty subjects were enrolled for this experiment. Each subject went through the screening procedures as outlined above, and randomized to receive one of the 4 proposed tPCS current intensities: 0.2 mA, 1 mA, 2 mA, and sham (a total of 10 per intensity condition). After consent and screening were obtained, participants completed the following:

- MMSE
- The Stroop test
- Word list memory task
- 64 channel high density array electroencephalography (EEG) – was collected by using the traditional methodology of eyes open (EO) and eyes closed (EC) for 10 minutes each; Subjects were sited in a comfortable position, a technician placed the previously soaked in potassium chloride solution the EEG geodesic net onto participant head. The recordings were done having the subjects in wake restful condition. Subjects were instructed to keep his/her focus in a fixed point marked on the wall in front of them. The EEG technician directed each subject to avoid excess of head movement, eye blinking, eye rolling, muscle tension or any other motion capable to produce non EEG artifacts on the recordings. During the EEG recordings the environmental noise was kept at minimum and participants were asked to keep their thoughts as neutral as possible (i.e.

avoiding thoughts about the monetary compensation or how much time was left into the experiment)

Stimulation: after initial baseline assessments, subjects underwent one session of tPCS, for 20 minutes at 2mA, at the current intensity to which they have been previously randomized (0.2 mA, 1 mA, 2 mA, and sham). Previous to the stimulation a research explained the procedure and described the feeling of the stimulation. A saline soaked pelt sponge over the stimulation electrode was mounted in each participant's ear lobe with an ear-clip. The Stimulation was ramped up during the first 30 seconds of the procedure and after this period the current intensity reached its maximum parameter, by the end of the stimulation period the current was ramped down for the final 30 seconds. Participants in the sham group received only the ramping periods at the beginning and at the end of the 20 minutes of sham stimulation.

After stimulation participants completed the above mentioned assessments for post-stimulation evaluation. Additionally, subjects completed:

- tPCS side effects questionnaire
- Blinding questionnaire

Statistical Analysis

The statistical analysis was done following the same software and methods described previously for experiment number one. As the main purpose of this experiment was to define the optimal current density as a parameter of stimulation, the statistical analysis used the variables for current density (0.2 mA, 1 mA, 2 mA, and sham) for statistical modeling.

3.3 Experiment 3 (cognitive behavioral and autonomic responses)

Participants

Thirty healthy volunteers were recruited to participate in this study; participants came from the Boston metropolitan area by posting ads in universities, colleges, public areas, and internet. Participants were eligible using the following inclusion criteria: age between 18 and 65 years; (2) no diagnosis of neurological, psychiatric, or metabolic disorder, as reviewed by a scientist physician; (3) no personal history of stroke, traumatic brain injury or epilepsy as reviewed by a scientist physician; (4) no drug or alcohol abuse; (5) no history of brain surgery or presence of metallic implants on the head; (6) pregnancy. All female participants were tested for gravidity using a urinary pregnancy test. A female researcher accompanied and performed the testing in the privacy of a restroom stall. All participants completed a Mini Mental State Examination (MMSE) as a brief screening tool to assess and determine neurocognitive capabilities, this with the purpose to rule out the presence of deficits or any other sign of deviant performance that could be indicative of a dementia problem. The MMSE is a well validated and standardized neurological scale used in the clinical and research settings for a quick assessment of cognitive impairment overtime (Reisberg, et al., 1982), although the MMSE is not used as primary outcome its main purpose was to be used as an objective measurement of safety. Each participant received \$50 US dollars as monetary compensation for participation.

All 30 participants that were recruited completed the single stimulation session. Half of the sample, 15 participants (50%) were randomized to the active group, while 15 (50%) were allocated to the sham stimulation group. There were no significant differences in demographic characteristics between groups, table 3.4 represents sample characteristics.

Demographics	Active Stimulation	Sham Stimulation
Male/female	7/8	6/9
Age, years	30.53 (7.59)	28.40 (5.15)

Table 3.4. Demographic data at baseline. Mean (SD)

Design

Experiment 3 was conducted as double-blind, sham-controlled randomized trial at the Neuromodulation Center, Spaulding Rehabilitation Hospital. We aimed to determine the effects of a single session of tPCS versus sham stimulation on performance on cognitive behavioral tasks and autonomic responses of hearth rate variability and electrodermal response. All participants provided written, informed consent. The experiment contained 2 arms, which were run in parallel between-subjects design. Participants were randomized into one of the two arms: sham stimulation or active stimulation with a random frequency ranging between 1-5 Hz, at 2 mA of current density for a period of 20 minutes. Each participant was allocated to one of the two groups and having one visit that included baseline recordings and assessments followed by exposure to intervention, and finishing with post intervention measurements. The independent variable for each arm was the stimulation intervention (active or sham) at which participant were allocated, the dependent variable were the results obtained as a main outcome (HRV, EDR, BART, and Arithmetic task), table 1 presents all of the dependent variables in the column named “assessments”, considering “time” as a factor each variable had two levels which were used for analysis; pre-stimulation and post-stimulation. Figure 3.4 shows the study design used for this experiment.

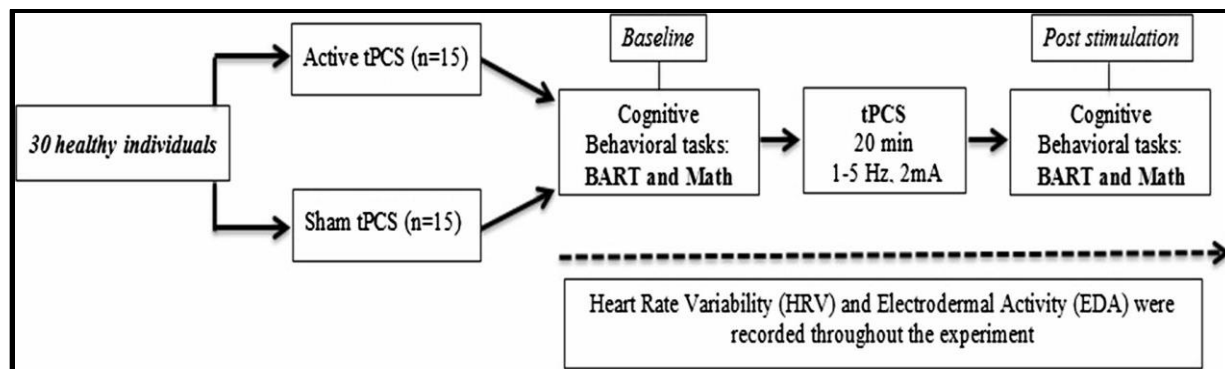


Figure 3. 4. Schematic representation of study design. From Morales-Quezada, *et al* 2014.

Materials Transcranial Current Pulsed Stimulation (tPCS)

All participants were randomized into one of two study arms. To create the randomization list a computer algorithm was used to generate permuted blocks of three (www.randomization.com). In all of the study arms, both the investigator assessing the outcomes and the participants were blind to the intervention. The tPCS device used was an investigational, custom-made, and battery powered. The same unit used for experiment one and two was used in this experiment, the tPCS device used for the active group used the following parameters; current intensity 2 mA, frequency; random ranging between 1-5 Hz, time 20 minutes of active stimulation. The device used for the sham condition was programmed to deliver electrical current just during the ramping up at the beginning of the session and after 20 minutes a ramping down current was delivered.

Physiological Assessments

Heart Rate Variability and Electrodermal response (EDR) were collected with Powerlab 26T as a signal acquisition system and the Labchart 8.1 software (ADInstruments, New South Wales, Australia). The two Ag-Ag-CL skin conductance electrodes were attached to the second and

third fingers of the non-dominant hand, between the first and second phalanges. EDR was analyzed offline using Labchart 8.1 (ADInstruments, New South Wales, Australia) as a mean value of 5 minutes of task performance. HRV was recorded using a 3-lead ECG with a lead I triangular configuration, sampling rate was 200 cycles per second. The HRV was acquired for 5 min before, during, and after task performance. After removing ectopic beats, HRV was analyzed offline using frequency domain measurements by using the HRV 2.0 module for Labchart (ADInstruments, New South Wales, Australia). Power Spectral Density (PSD) analyses for short term recordings were processed using FFT, three main spectral components were distinguished and used for analysis; very low frequency (VLF), low frequency (LF), and high frequency (HF) components, and also a ratio of LF/HF was calculated as a marker of autonomic modulation. Both HRV and EDR are considered markers of autonomic function (Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), the balance between sympathetic and parasympathetic responses are mediated by central autonomic centers, when subjects are exposed to stressful conditions the sympathetic system releases noradrenergic neurotransmitters which have the effects of decreasing HRV while EDR increases, contrasting with the effects of the parasympathetic system which increase HRV and decrease EDR. Variations in these physiological parameters during cognitive testing offer important evidence of how an individual adapts to stressful conditions, moreover, it can also be sensible to detect physiological fluctuations facilitated by tPCS applications.

Mini Mental State examination (MMSE)

As in the case of experiment number one the MMSE was used as measurement of safety.

Neuropsychological Assessments

For this experiment the Stroop test was applied with the same purpose as experiment one and two, The Stroop test primary purpose was as measurements of safety.

Balloon Analog Risk Task (BART)

The BART consisted of a gambling paradigm as a measurement of decision-making behaviors and it is able to evaluate higher frontal and prefrontal executive functions (Fecteau et al., 2007), the task was designed based on the assumption that rewards to the subject in a gambling environment, leads to a risk behavior paradigm where the subject needed to either to inhibit his/her the impulses or to keep gambling with the expectation to win over the risk to lose everything (Figure 3.5). The riskiness on the BART has been correlated with self-reported occurrence of addictive, health, and safety risk behaviors, with the task accounting for variance in these behaviors beyond that accounted for by demographics and self-report measures of risk-related constructs (Lejuez et al., 2002). The outcome measures for this experiment were as follows:

- a) **Choice of low risk vs. High risk in each trial.** This outcome was measured as the percentage of cases in which the subject chose the option with safer or higher probabilities (higher chances of guessing the right boxes, but earning less points) it will be considered as a binary variable.
- b) **Time elapsed between presentation of the possibilities and decision, measured in milliseconds, considered as a continuous variable.** Performance for each task will be measured, excluding those that have equal probabilities for each color (3:3 ratios).

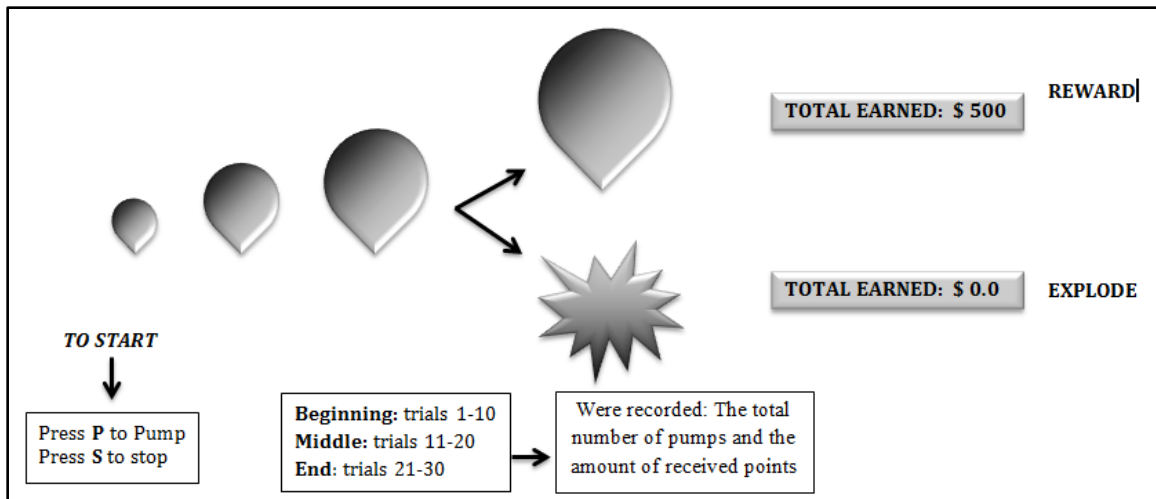


Figure 3. 5. Balloon analog risk task. Subjects engaged in this task keep pumping the balloon up to the point they decide to stay the earned amount, if risk behavior is continue, the balloon explodes and all earned points are lost. From of Morales-Quezada, *et al* 2014.

Arithmetic Task

This task involves the evaluation of cognition pathways for the processing of mental arithmetic . The task was generated using the SuperLab software (Cedrus Co, San Pedro, CA.) and presented to the subjects in an easy understandable manner. Subtractions were used as the main arithmetic procedure and it was divided into three levels of complexity: easy, intermediate and difficult, which were randomized through the duration of the experiment. The outcomes of this task were accuracy (those who are more accurate complete more tasks in the same amounts of time than those who are not as accurate) and number of correct answers (Figure 3.6). The utilization of this task was with the intention to explore the effects of tPCS on cognitive modulation of mathematical processing. There is consistent evidence associating parietal, temporal and prefrontal structures with arithmetic performance (Grabner et al., 2009). Based on computer modeling of current distribution, it is possible to reach temporal-parietal circuits involved in the processing of arithmetic calculations. The task is sensitive to detect changes in performance and can provide understanding of how tPCS exerts its influence on higher cortical functions.

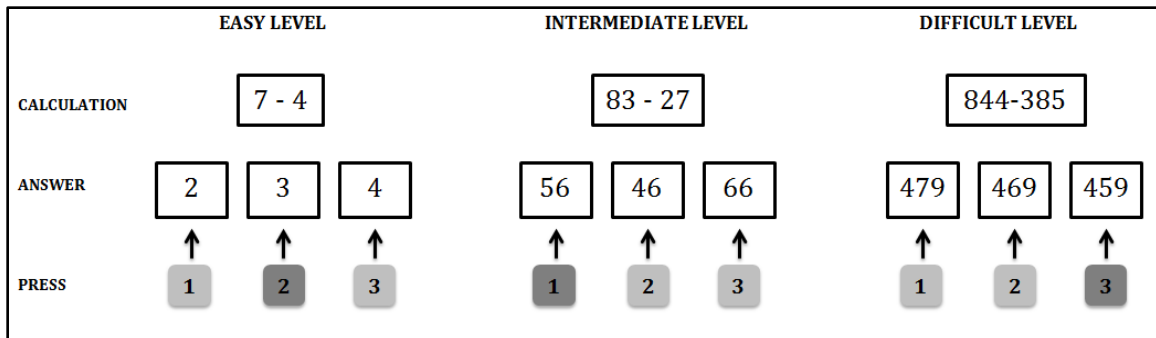


Figure 3. 6. Arithmetic task and its three levels of difficulty, each of the keys represented one of three possible answer options. From Morales-Quezada, *et al* 2014.

Study Procedures

Thirty subjects were enrolled for this experiment. Each subject went through the screening procedures as outlined above, participants were randomized to receive either active tPCS or sham tPCS (total 15 subjects per condition), using the optimal parameters (frequency and intensity) determined in experiments 1 and 2. After consent and screening were obtained, participants completed the following:

- MMSE
- Stroop Task
- HRV: Participants were sited in a comfortable position, electrocardiograms were recorded using a 3-lead ECG with a lead I triangular configuration, participants went to a period of relaxed sitting position for 5 minutes in order to reach a restful baseline condition before the ECG was recorded.
- EDR: two Ag-Ag-CL skin conductance electrodes were attached to the second and third fingers of the non-dominant hand, between the first and second phalanges, as in the case

of the HRV, the EDR was recorded after a period of relaxed state for the baseline measurement.

- **BART:** Before and after stimulation, subjects performed the RBT consisting of a gambling paradigm as a measurement of decision-making behaviors. Participants were sited in front of a computer monitor allowing them to adopt a comfortable position between their arms and the keyboard, the task was presented in a 24” display size computer monitor.
- **Arithmetic Task:** Before and after stimulation, participants performed the arithmetic task consisting in a subtraction operations divided in three different levels; easy one digit arithmetic operation, medium two digits arithmetic operation, and difficult three digits arithmetic operation presented randomly to the subject. Participants were sited in front of a computer monitor allowing them to adopt a comfortable position between their arms and the keyboard, the task was presented in a 24” display size computer monitor.

Stimulation: Subjects received one session of tPCS, either active (1-5 randomly oscillating frequency at 2 mA for 20 minutes) or sham stimulation.

After stimulation the subject will complete the above pre-stimulation assessments again.

Additionally, subjects will complete:

- tPCS side effects questionnaire
- Blinding questionnaire

Statistical Analysis

The statistical analysis was done following the same software and statistical methods described previously for experiment number one and two. For the arithmetic task the variation in

accuracy defined as pre to post experimental condition was calculated using the following formula: $\frac{\text{Post-pre accuracy}}{\text{pre accuracy}} \times 100$. Independent sample t test were used to compare the mean differences between groups for the simple and the complex arithmetic level. Additionally, exploratory subgroup analyses were conducted using paired-sample t test to compare for mean differences in accuracy from pre to post between the active and sham group. The percentage of variation for the BART task was calculated using the previously described formula for the arithmetic task: $\frac{\text{Post-pre accuracy}}{\text{pre accuracy}} \times 100$. To compare the mean differences in total points earned and variation in the average number of pumps, the independent sample t test was used. Given the need to add one additional variable for analysis [block-by-block analysis] the mixed ANOVA model with *block* as the within-subject factor (with three levels: 1-10; 11-20; 21-30) and *group* as the between-subject factor (with two levels: active or sham).

HRV frequency domain variables included: total power $\approx \leq 0.4$ Hz, (2) very low frequencies ≤ 0.04 Hz (*VLF*), (3) low frequencies 0.04- 0.15 (LF), (4) high frequencies 0.15- 0.4 Hz (HF), and (5) LF/HF ratio. Analysis was performed by HRV frequency band using unpaired t test. To evaluate for correlations between changes in HRV and performance in the arithmetic and BART tasks, a Spearman's rank correlation between HRV parameters and tasks performance in both active and sham groups were used.

EDR was analyzed pre and post stimulation (during task performance and throughout the stimulation period). A mixed ANOVA model with *time* as within-subject factor (pre and post) and *group* as the between-subject factor (active or sham tPCS) where used for statistical computation, results were considered significant if $p < 0.05$. For significant results, measures of effect size were calculated for ANOVA, and are presented as the correlation between an effect and the dependent variable. If the value of the measure of association is squared it can be

interpreted as the proportion of variance in the dependent variable that is attributable to each effect. For the analysis of this experiment the eta squared (η^2) was used.

Further analysis was used applying two-way ANOVA to compare mean differences between groups, by analyzing two independent variables (factors). The purpose of a two-way ANOVA was to observe if there was interaction between the two independent variables on the dependent variable.

3.4 Experiment 4 (cognitive behavioral and autonomic responses)

Participants

Thirty healthy volunteers were recruited to participate in this study; participants came from the Boston metropolitan area by posting ads in universities, colleges, public areas, and internet.

Participants were eligible using the following inclusion criteria: age between 18 and 65 years; (2) no diagnosis of neurological, psychiatric, or metabolic disorder, as reviewed by a scientist physician; (3) no personal history of stroke, traumatic brain injury or epilepsy as reviewed by a scientist physician; (4) no drug or alcohol abuse; (5) no history of brain surgery or presence of metallic implants on the head; (6) pregnancy. All female participants were tested for gravidity using a urinary pregnancy test. A female researcher accompanied and performed the testing in the privacy of a restroom stall. All participants completed a Mini Mental State Examination (MMSE) as a brief screening tool to assess and determine neurocognitive capabilities, this with the purpose to rule out the presence of deficits or any other sign of deviant performance that could be indicative of a dementia problem. The MMSE is a well validated and standardized neurological scale used in the clinical and research settings for a quick assessment of cognitive impairment overtime (Reisberg, et al., 1982), although the MMSE is not used as

primary outcome its main purpose was to be used as an objective measurement of safety. Each participant received \$50 US dollars as monetary compensation for participation.

All 30 participants that were recruited completed the single stimulation session. Half of the sample, 15 participants (50%) were randomized to the active group, while 15 (50%) were allocated to the sham stimulation group. There were no significant differences in demographic characteristics between groups, table 3.4 presents sample characteristics.

Demographics	Active Stimulation	Sham Stimulation
Male/female	8/7	6/9
Age, years	27.53 (3.59)	25.83 (2.15)

Table 3. 4. Demographic data at baseline. Mean (SD)

Design

Experiment 4 was conducted following the same design described for experiment 3, as double-blind, sham-controlled randomized trial at the Neuromodulation Center, Spaulding Rehabilitation Hospital. We aimed to determine the effects of a single session of tPCS versus sham stimulation on performance on cognitive behavioral tasks and autonomic responses of hearth rate variability and electrodermal response. All participants provided written, informed consent. The experiment contained 2 arms, which were run in parallel between-subjects design.

Participants were randomized into one of the two arms: sham stimulation or active stimulation with a random frequency ranging between 1-5 Hz, at 2 mA of current density for a period of 20 minutes. Each participant was allocated to one of the two groups and having one visit that included baseline recordings and assessments followed by exposure to intervention, and finishing with post intervention measurements. The independent variable for each arm was the

stimulation intervention (active or sham) at which participant were allocated, the dependent variable were the results obtained as a main outcome (HRV, EDR, Attention Switching Task, and Paired associate Learning task), table 1 presents all of the dependent variables in the column named “assessments”, considering “time” as a factor each variable had two levels which were used for analysis; pre-stimulation and post-stimulation. Figure 7 shows the study design used for this experiment.

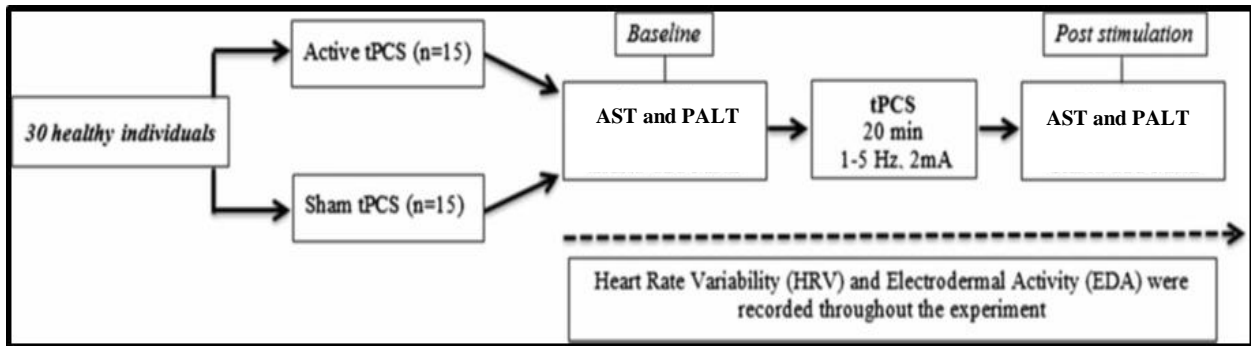


Figure 3. 7. PALT and AST design Study design. Paired Associative Learning Task (PALT) and Attention Switching Task (AST).

Materials

Transcranial Current Pulsed Stimulation (tPCS)

The same unit used for experiment one and two was used in this experiment, the tPCS device used for the active group used the following parameters; current intensity 2 mA, frequency; random ranging between 1-5 Hz, time 20 minutes of active stimulation. The device used for the sham condition was programmed to deliver electrical current just during the ramping up at the beginning of the session and after 20 minutes a ramping down current was delivered.

Physiological Assessments

HRV and EDR were obtained and recorded as previously described in experiment 3.

Mini Mental State examination (MMSE)

The MMSE was used and followed the same methodology described in previous experiments.

Neuropsychological Assessments

For this experiment the Stroop test was applied with the same purpose as described in previous experiments. The Stroop test primary purpose was as measurements of safety.

Attention Switching Task (AST)

The AST is a method to examine the demands on information-processing capacity; it indicates that switching attention per se between input channels does not constitute the primary demand for central processing capacity in dichotic tasks. The increased demand appears to be related instead to the concomitant changes required in the input processing and to the processing of signals to switch the focus of attention (Laabs & Stager, 1976). The task was design using the SuperLab software (Cedrus Co, San Pedro, CA.) and consisted in 4 sections (see figure 8). Each section has 64 trials (group of letters or numbers) blocks. Each trial started with a cue presented on the computer screen for 500 msec, which was then replaced by a 2000 msec target. Before each stimulus a cue is shown, it could be a + or a Δ. The + cue refers to number of letter; if the stimuli presents a pair number of letters (2 or 4), the participant should press “A” key and if the stimuli consisted of even number of letters (3 or 5) the participant should press “L” key. The Δ character refers to the type of letter; if the stimuli presented vowel letter, the participant should press “A” key and if the stimuli presented consonant letters, the participant should press “L” key.

The first section consists of 32 pair/even stimuli followed by 32 vowel/consonant stimuli; the second section consists of 32 vowel/consonant stimuli followed by 32 pair/even stimuli; the

third section presents randomly 32 pair/even and 32 vowel/constant stimuli but with an extra cue; the letter are between <> if the stimuli are pair or vowel and the letter are between >< if the stimuli are even or consonant. The fourth section presents randomly 32 pair/even and 32 vowel/consonant stimuli. Each experiment takes 2-3 minutes to answer. The outcome measures for the AST included: Reaction time, accuracy and error rates, and switch cost can be recorded and analyzed, where procedural and semantic properties of learning and memory can be explored using this particular task, performance, attention and the ability to manipulate information can be assessed as well by the SuperLab software.

Paired-Associate learning test (PAL)

The paired words task was design using the SuperLab software (Cedrus Co, San Pedro, CA.), described by Ingris (1959), is a quick test to assess declarative memory and learning with the use of cues. It is validated it for the assessment of memory impairment in elderly patients and since then it has been used extensively in studies addressing declarative memory changes in healthy subjects. The outcome measurements for the PAL included: Number of trials to achieve 60% accuracy, the number of correct responses in the first and in the last trial and the difference between the number correct in the first and last trial (improvement). This task consisted of two phases - learning and recall. During the learning phase, word pairs were randomly presented to the participant in the center of the computer screen with a 0.6° visual angle. Participants were given one set of 20 word pairs and were instructed to read the words and repeat them to themselves as many times as they could during 5 seconds. Each pair was presented for 5 seconds, with an inter-pair interval of 500msec. After all 20 word pairs were presented, participants were instructed to complete an incomplete word pair by writing the missing word (recall period).

Participants were given 20 word stems, randomly presented on a computer screen, and they were asked to write the missing word to complete the word pair. The duration of each stem word was self-passed and the order of the words was completely randomized (see figure 8). The main outcome for this task was accuracy.

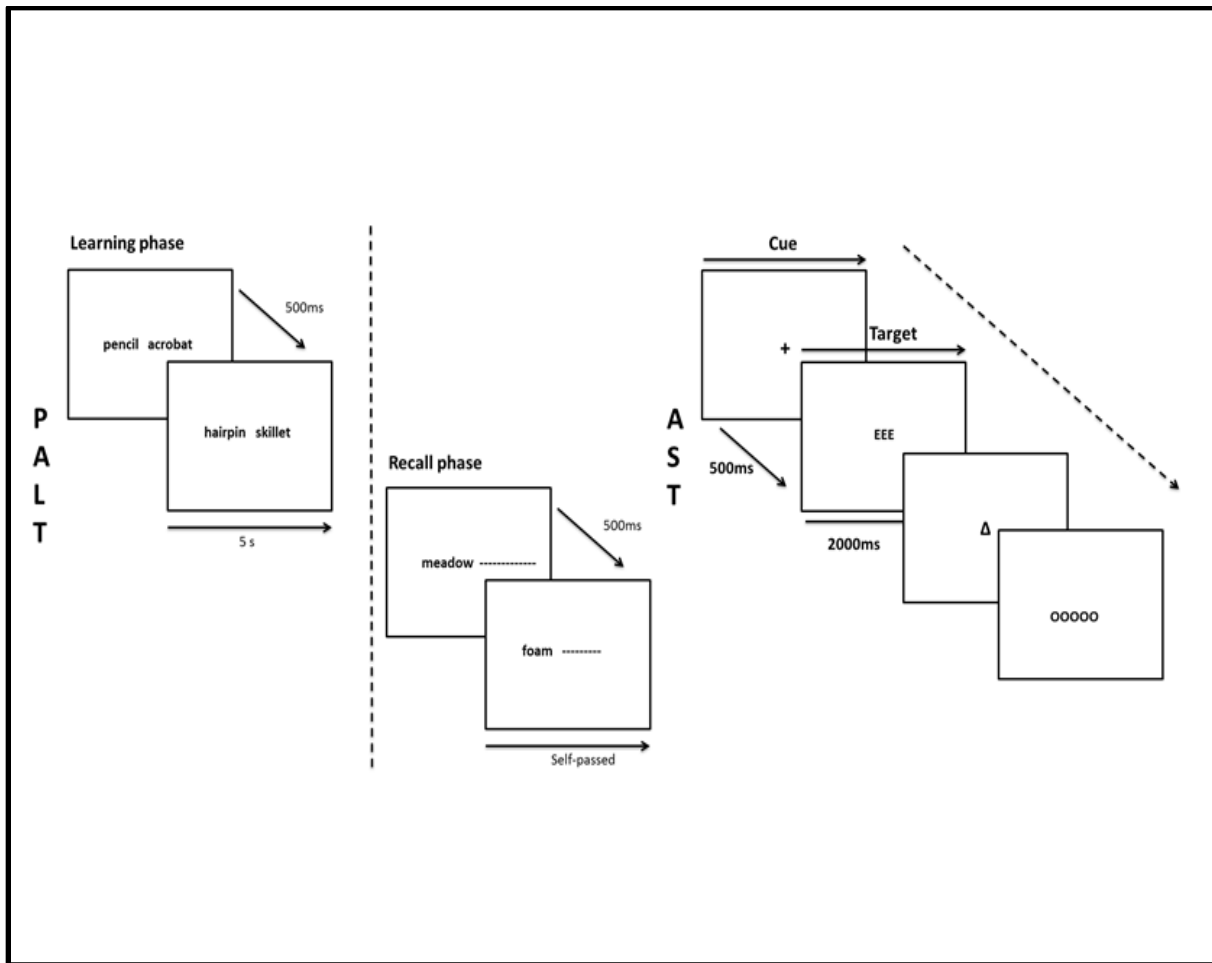


Figure 3. 8. Cognitive task Design. Paired Associative Learning Task (PALT) showing the time interval between stimuli presentation (500ms) and stimuli exposure (5 sec) during the learning phase, the recall phase was self-paced. Attention Switching Task (AST) with presentation of the stimuli (2000ms) and interval of 500ms in between stimuli.

tPCS side effects questionnaire and tPCS blinding questionnaire

Both questionnaires were used to evaluate the presence of any adverse effect related with the intervention and to validate the blinding method, the questionnaires were applied following the same approach described in previous experiments.

Statistical Analysis

Descriptive statistics were initially performed to evaluate the normality of the data by using the Shapiro-Wilk test and by estimating skewness and kurtosis. We then used a mixed model ANOVA with *group* (active vs. sham tPCS) as the between subject factor and *time* (pre vs. post) as the within subject factor to analyze the effects of pre and post tPCS in: a) accuracy on the PALT; b) Response time (RT) on the AST; c) Accuracy on the AST; d) EDR in the PALT; and e) EDR in the AST. Heart rate variability measurements were the same as previously used in experiment 3. We also used an ANOVA model to analyze the changes for each HRV frequency band. When sphericity was not met, the Greenhouse-Geisser correction was applied to the degrees of freedom in all cases with the corrected probabilities. Post hoc comparisons of the mean values were conducted by paired comparisons, using Bonferroni correction for multiple comparisons. For statistical significant results, measures of effect size were calculated for ANOVA, and are presented as the correlation between an effect and the dependent variable. If the value of the measure of association is squared it can be interpreted as the proportion of variance in the dependent variable that is attributable to each effect. For the analysis of this experiment the eta squared (η^2) was used.

Further analyses were applied by two-way ANOVA to compare mean differences between groups, by analyzing two independent variables (factors). The purpose of a two-way ANOVA

was to observe if there were interactions between the two independent variables on the dependent variable.

4. RESULTS

This section contains the results generated from the experiments performed as a part of this research on tPCS. Each experiment results are presented separately and in accordance with the research hypothesis and aims. The initial aim of this investigation was to determine which tPCS parameters; stimulation bandwidth frequency and current intensity can elicit measurable quantitative changes in the properties of the EEG bands. *The hypothesis is that low-frequency, low-current intensity of application will not generate much change in the qEEG analysis, while higher frequencies and current intensities will evoke EEG band shifts on the qEEG power, qEEG coherence and mean frequency analysis.* Once evaluated the optimal parameters, neuropsychological and physiological functions were also explored with the intention to observe any specific tPCS modulations had on these domains.

4.1 Experiment 1 (definition of frequency)

The main research question for this experiment focused on the frequency (Hz) of stimulation. Could different parameters of tPCS frequencies, would lead to differential changes in the neurophysiology as measured by qEEG? And if so, what would be the impact of these changes on cognitive and behavioral tasks?

For experiment 1 main aim was to determine which tPCS parameters; specifically, by applying different frequencies, elicited a measurable qEEG change in the power and mean frequency of

the EEG bands. *The hypothesis established that low-frequency and high fixed frequency ranges (1Hz and 100Hz respectively) , will not generate much change in the qEEG analysis, while random pulsed frequencies(1 to 5Hz randomly pulsed) will evoke EEG band shifts on the qEEG power and mean frequency analysis.*

Out of the 40 participants that were recruited, 38 completed the single stimulation session. Two subjects signed the consent form and decided not to participate before stimulation was given.

Nine participants (23.68%) were randomly allocated to the sham group, 9 (23.68%) to the 1 Hz stimulation group, 9 (23.68%) to 100 Hz stimulation, and 11 (28.95%) to 1-5 Hz random frequency stimulation. One subject in the 1-5 Hz random frequency group was removed from the qEEG analysis due to excessive artifacts in the EEG recording.

In the analysis for behavioral monitoring and adverse effects, a one-way ANOVA using the difference between the post and pre-experimental condition between groups was used; when the assumption of sphericity in the data was not met a Greenhouse correction was applied. The results for this experiment showed no effects of stimulation for any dependent variables tested with the Stroop test: response time for colors ($F(1,28) = 18.872, p = 0.5$), response time for words ($p = 0.28$), response time for word and color interference ($F(1,28) = 18.872, p = 0.27$) and number of correct words–word memory task ($F(1,28) = 18.872, p = 0.38$). There were no significant differences in the incidence of adverse effects between groups ($F(1,28) = 25.038, p = 0.751$). All of the reported side effects were mild and consisted of redness and pain in the area of the electrodes location, tingling sensation and itching were the most reported side effects (100% of participants in both groups), while a mild headache was reported by only 1 subject (2.63%).

The results obtained in these evaluations showed that tPCS has not acute detrimental effects in memory and attention.

QEEG coherence analysis and interhemispheric functional connectivity were explored by selecting the pair of recording electrodes close to the area of stimulation, as is well known the influence of the stimulation will be stronger in the area immediately under the stimulating electrodes. The measurements with significant results were observed for the pair of electrodes; FT9-FT8 and AF7-AF8. A mixed model ANOVA was used to evaluate changes in interhemispheric coherence level for each frequency band and sub-band. No statistical significant effects were seen for the interaction term *eyes condition* \times *group* for any of the EEG frequency bands (alpha $p = 0.724$ and theta $p = 0.801$) or sub-bands (low-alpha $p = 0.683$, high-alpha $p = 0.79$, low-beta $p = 0.467$ or high-beta $p = 0.255$), nor for the low-beta ($p = 0.896$) and high-beta ($p = 0.577$) sub-bands. No significant effect on *eyes* as a factor was seen for any of the EEG frequency bands or sub-bands.

A significant effect of group was found for the alpha band ($p = 0.022$) with an effect size (ES) of $\eta^2 = 0.137$, low-alpha band ($p = 0.0169$) (ES $\eta^2 = 0.145$) and theta band ($p = 0.0109$) (ES $\eta^2 = 0.234$). Post-hoc comparisons with effects of the intervention on interhemispheric coherence for each frequency band used a mixed model analysis of variance using coherence difference between the post and pre-experimental conditions. Theta interhemispheric coherence showed that the active treatment with random frequency differed significantly from sham ($p = 0.0284$) (ES $\eta^2 = 0.397$), reflecting a significant increase in theta coherence for the random frequency group (mean difference = 0.089 points, 95% CI 0.01 to 0.1693). No significant effects were found between sham and 100 Hz ($p = 0.37$) or sham and 1 Hz stimulation ($p = 0.76$). Further exploratory analysis showed that the theta interhemispheric coherence was significantly higher

for the random frequency group compared to 1 Hz ($p = 0.038$) (ES $\eta^2 = 0.347$) and 100 Hz stimulation ($p = 0.0013$) (ES $\eta^2 = 0.429$).

Post-hoc analysis for interhemispheric coherence for the low-alpha band after stimulation with random frequency showed a trend for a significant increase in connectivity compared to sham ($p = 0.05$) (ES $\eta^2 = 0.098$), reflecting a coherence increase in 0.09 points (95% CI -0.0002 to 0.18). There were no significant observed differences between the 100 Hz stimulation and sham intervention ($p = 0.67$) or stimulation at 1 Hz and sham ($p = 0.18$) for this specific frequency band. No significant differences were found for alpha coherence between the active random frequency group and sham ($p = 0.1520$) or 100 Hz and sham ($p = 0.39$). 1 Hz stimulation showed a tendency toward decrease of alpha coherence compared to sham ($p = 0.093$) of 0.06 points (95% CI -0.13 to 0.012) (Figure 1).

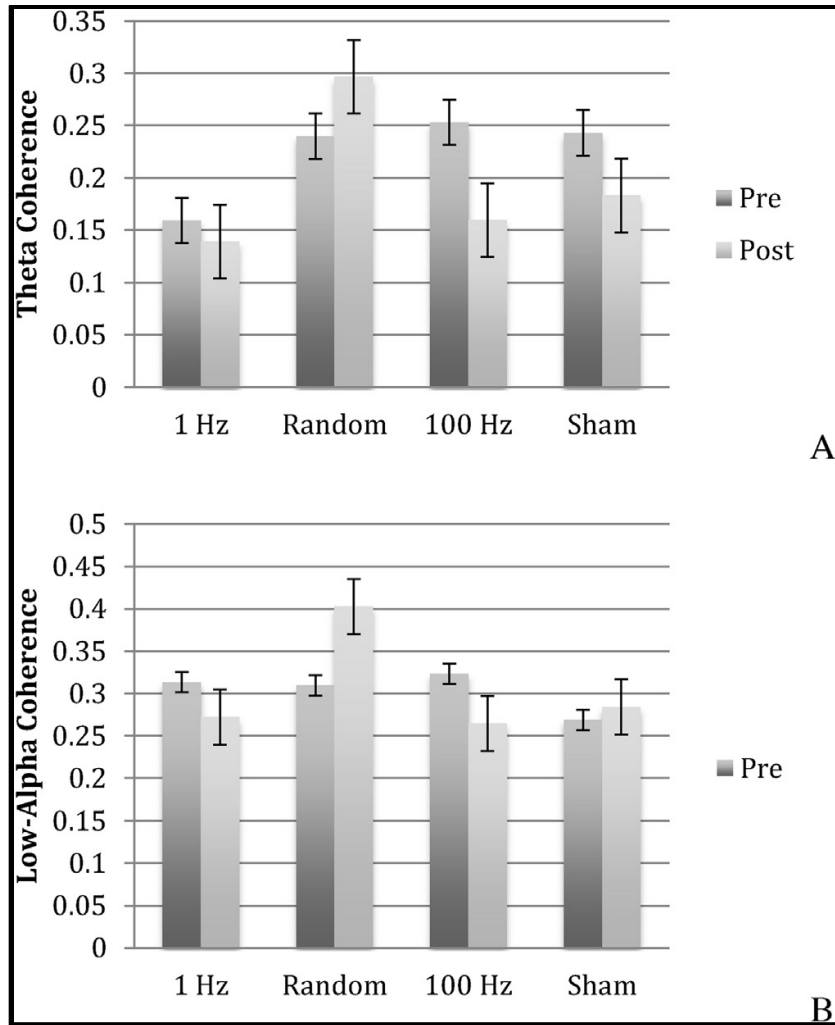


Figure 5 1. Effects of tPCS on theta (A) and low-alpha (B) coherence. MS coherence is a measure of how well x is correlated to y on EEG metrics is considered a measure of connectivity, the random frequency stimulation proved to be more effective in modulating coherence. Bars represent \pm 1SE.

The analysis of the EEG power was done by looking for changes in the mean power for each frequency bands by using a mixed model ANOVA. No significant changes were found for the interaction $eyes \times group$ for theta power ($p = 0.4753$), alpha power ($p = 0.834$) or beta power ($p = 0.404$). No significant effect for group was found for either the theta band ($p = 0.29$) or the beta band ($p = 0.43$). A significant effect for group was found for the alpha band ($p = 0.044$).

Post-hoc comparisons with showed a significant decrease of alpha mean power for 1 Hz stimulation compared to sham ($p = 0.0445$) (ES $\eta^2 = 0.0997$) of 0.183 points (95% CI 0.0048 to

0.362). Further exploratory analysis showed that this difference was also significant when compared to the random frequency group ($p = 0.034$) (ES $\eta^2 = 0.138$), but not when compared to 100 Hz stimulation ($p = 0.212$). No significant effects were found for *eyes* as a factor for any of the analyzed bands.

Participants did not guess correctly the stimulation group to which they were assigned beyond chance ($p = 0.679$).

4.2 Experiment 2 (defining stimulation intensity)

The main objective for this experiment was to evaluate the effects of different tPCS current densities on qEEG analysis (0.2mA, 1mA, 2mA, and sham). 40 participants were recruited, one volunteer decided not participate before randomization was completed; only 39 participants completed the single session stimulation. A participant from the 2 mA group and another from the 0.2 mA group were removed from the qEEG analysis due excess of artifact in their recordings. No significant differences in demographic characteristics were observed among groups (table 3 methods section).

The hypothesis behind this experiment was that stimulation with 2 mA would enhance interhemispheric coherence for the low-frequencies bands compared with sham stimulation, 1mA intensity would induce similar effects in a lesser magnitude, and low-intensity stimulation would not have significant effects when compared with sham. To evaluate the effects of 2 mA stimulation versus sham on coherence difference (post-experimental – pre-experimental condition) for each frequency band, an unpaired one-sided t-tests was used. The same analysis was performed to assess each group versus sham.

In accordance with the hypothesis the coherence analysis showed a significant increase for the theta band coherence compared with the sham intervention ($p = 0.0166$) (ES $\eta^2 = 0.0577$) reflecting a mean increase of 0.094 points (SE = 0.042). No significant differences were found for the alpha band ($p = 0.162$) or high-alpha sub-band ($p = 0.183$) coherence. The low-beta and high-beta sub-bands showed a significant increase in coherence ($p = 0.0336$ and 0.032 , respectively) (ES $\eta^2 = 0.0341$ and $\eta^2 = 0.0327$). Although changes in low-alpha coherence were not statistically significant ($p = 0.149$), there was a trend toward increase that was clear when compared with the sham stimulation (Figure 2). These results replicated and confirmed the results from experiment number 1 where a random frequency ranging between 1-5 Hz increased theta interhemispheric coherence.

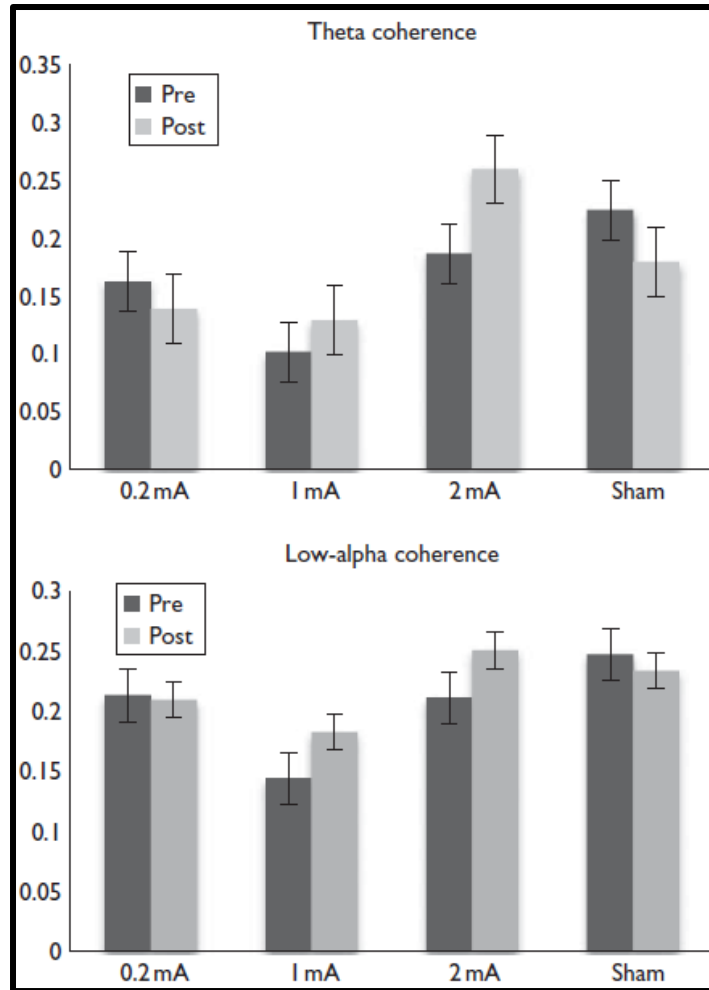


Figure 5.2. Interhemispheric coherence of theta and low alpha. Interhemispheric coherence for the theta and low-alpha frequency bands. Bars represents SE. With permission from (Morales-Quezada)

Stimulation with 1mA also showed a significant increase for theta coherence compared with the sham group ($p = 0.0338$) (ES $\eta^2 = 0.0471$), reflecting a mean increase of 0.081 points (SE =0.043). No significant differences were found for the high-alpha ($p =0.086$), low-beta ($p =0.339$), and high-beta sub-band ($p = 0.339$). There was a trend for coherence increase for both alpha ($p = 0.0682$) and low-alpha ($p = 0.0587$), reflecting a mean increase of 0.073 points (SE = 0.048) and 0.079 points (SE= 0.049), respectively. Stimulation with 0.2 mA showed no significant differences when compared with the sham group in interhemispheric coherence for

alpha ($p = 0.332$), theta ($p = 0.26$), low-alpha ($p = 0.226$), high-alpha ($p = 0.47$), and high-beta ($p = 0.224$). When compared with 2 mA stimulation, the 1mA group showed no significant differences for coherence of any of the bands or sub-bands analyzed. Based on these results we can conclude that stimulation with 1mA is not different than 2mA stimulation and increases theta coherence when compared with sham stimulation. An interesting observation in the analysis was that low intensity stimulation at 0.2 mA group behaved somewhat as the sham group in decreasing interhemispheric coherence.

To assess the modulation in mean power for each EEG frequency band, we used a mixed model analysis of variance with the dependent variable being the power difference between the post-experimental and pre-experimental condition with the following independent variables: a within-subject variable for eyes condition (two levels – open and closed), a between-subject variable of group (four levels), and the interaction term (eyes \times group). The quantitative analysis of electroencephalographic power showed no significant effects of group for any of the EEG frequency bands. [Theta power ($p = 0.415$), alpha power ($p = 0.415$)], or any of the sub-bands [low-alpha ($F_{73,3}=0.33$, $p = 0.802$), high-alpha ($p = 0.468$), low-beta ($p = 0.4753$), high-beta ($p = 0.4753$)]. No significant changes were found for the interaction eyes \times group or for the eyes term for any of the frequency bands.

For the analysis of behavioral outcomes we used a one-way ANOVA for the difference between the post-experimental and pre-experimental condition between groups, the Greenhouse correction was applied when the assumption of sphericity was not met. No significant effects of stimulation for any of the dependent variables tested in the Stroop task: response time for colors ($p = 0.71$), words ($p = 0.31$), and word and color interference ($p = 0.76$). No significant effects were found for performance on the word memory task ($p = 0.18$).

For validation of blinding, the Fisher's exact test was used to assess differences between the four groups. Participants did not guess correctly the intervention group to which they were assigned ($p = 0.693$). No significant differences were observed in reporting adverse effects between groups ($p = 0.751$), all of the mentioned adverse effects were mild and involved redness and tingling sensation in the area of the electrodes placement.

4.3 Experiment 3 (cognitive behavioral and autonomic responses)

Once the stimulation parameters were characterized, experiment 3 was designed to explore the effects of tPCS on cognition and physiological responses. This experiment aimed to determine the effects of a single session of tPCS versus sham stimulation have on performance, both cognitive and behavioral functioning were assessed using specific standardized tasks. Autonomic responses of hearth rate variability and electrodermal activity were monitored to observe if active stimulation was able to modulate stress response. The hypothesis was to determine if active tPCS improved cognitive behavioral performance on the arithmetic and risk behavior tasks and that is superior to sham tPCS. 30 subjects were enrolled to participate and all of them completed the single session of stimulation. Two subjects were removed from HRV analysis (one in the active group and another from the sham group) due to excess of artifact in their recordings.

Arithmetic task

For this task the percentage of variation in accuracy (from pre- to post-experimental condition) was calculated using the following formula: $\frac{\text{Post-pre accuracy}}{\text{pre accuracy}} \times 100$. Independent sample t tests were used to compare the mean between groups for the simple and the complex level.

Additionally, exploratory subgroup analyses were conducted using paired-sample *t* tests to compare the mean difference in terms of accuracy from pre to post between the active and sham group. The analysis for the arithmetic task was conducted by level of the task. For the simple level (one digit operation), there were no significant differences between the two groups (mean difference: 0.114, SE: 2.387) ($t = 0.048$, $p = 0.962$). For the complex-level task no differences were observed across groups (mean difference = 3.882, SE = 3.664) ($t = 1.060$, $p = 0.299$), exploratory subgroup analysis showed that active tPCS was able to significantly increase performance from the pre to the post condition and only in the complex level (three digits) of mathematical calculation, with a mean difference of 5.458 and SE of 2.231 ($t = 2.446$, $p = 0.029$) (ES = 0.0423) (figure 3). This effect was not observed for the sham tPCS group.

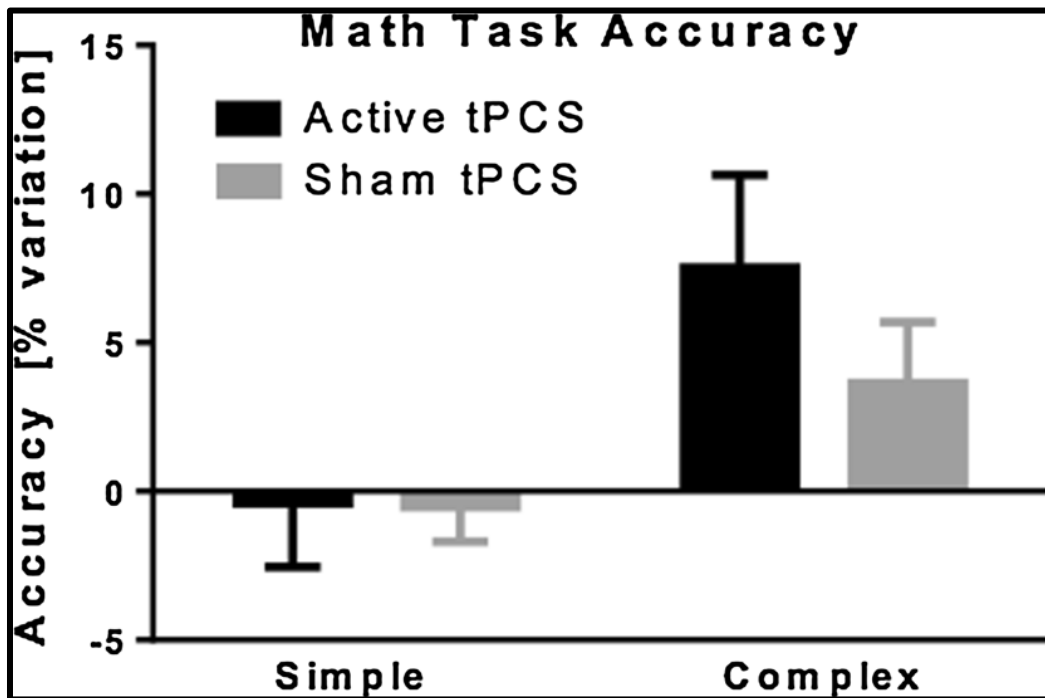


Figure 5 3. Arithmetic task. Accuracy results for the arithmetic task. With permission (Morales-Quezada)

BART

For the BART task, the percentage of variation was calculated using the following

formula: $\frac{\text{Post-pre accuracy}}{\text{pre accuracy}} \times 100$. Independent sample t tests were used to compare mean

differences in total points earned and variation in average number of pumps. Because the need to

add one additional variable a block-by-block analysis was additionally conducted two mixed

model ANOVAs with *block* as the within-subject factor (with 3 levels: 1–10; 11–20; 21–30) and

group as the between-subject factor (with 2 levels: active or sham tPCS). No significant

differences were observed between active and sham groups for total earned points (from pre to

post), reflecting a mean difference of -6.723, and SE of 11.360 ($t = -0.592, p = 0.559$) (figure 4).

There were no significant effects for the average number of pumps (mean difference = -2.017, SE = 11.951) $t = -0.169, p = 0.867$. No significant main effects or interactions were found in the block-by-block analysis.

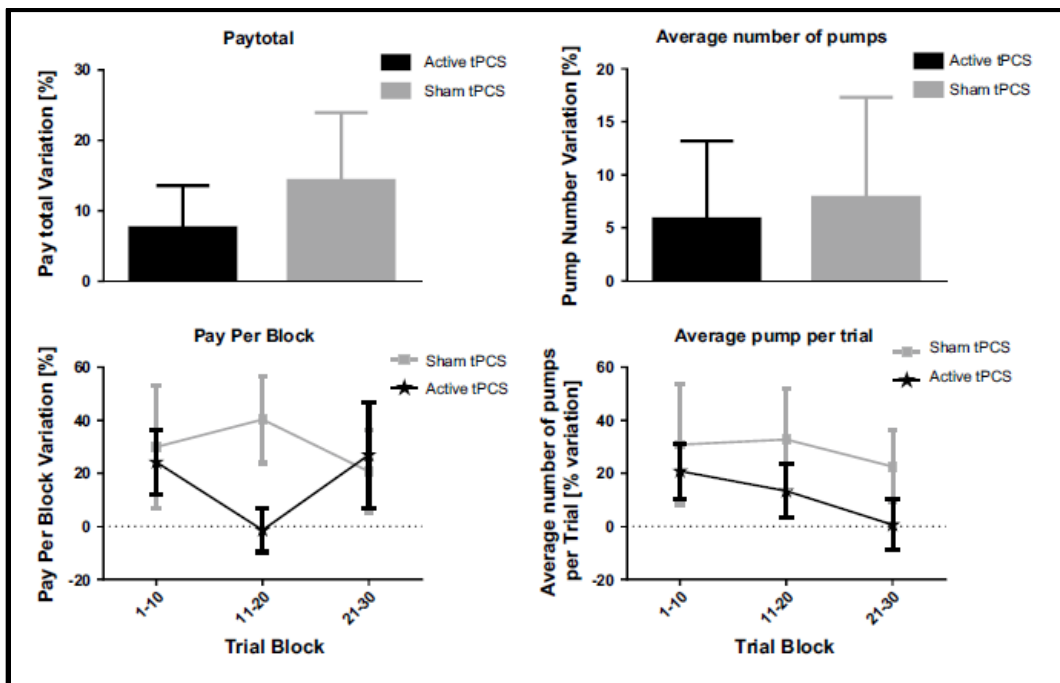


Figure 5. 4. BART task. Total pay and average number of pumps for the BART task are depicted. With permission (Morales-Quezada

Stroop Task

The mixed model ANOVA showed a main effect of *congruency* ($F(1,28) = 18.872, p < 0.001$), (ES $\eta^2 = 0.1964$) and *time* ($F(1,28) = 25.038, p < 0.001$) (ES $\eta^2 = 0.1243$). No effects were found for the interaction factors *congruency* \times *group* ($F(1,28) = 1.340, p = 0.257$), or *congruency* \times *time* \times *group* ($F(1,28) = 0.004, p = 0.952$). Meaning that overall, participants were answering faster during the trials with congruent stimuli ($M = 1,035.889, SE = 37.851$) than during trials with non-congruent stimuli ($M = 1,194.668, SE = 64.369$) ($p < .001$). They were also faster performing the second time they performed the task ($M = 1,013.135, SE = 42.951$) than during the first trial ($1,217.422, SE = 62.423$) ($p < 0.001$), representing somewhat a phenomenon of learned practice during this component of the task.

Physiological responses

For the Heart Rate Variability unpaired *t* test showed a significant increase in HRV total power for the active group when compared to sham ($p = 0.05$) (figure 5a), reflecting a mean increase of 824 units (SE = 72.78) (figure 5 b). The LF/HF ratio showed a significant decrease in the active group ($p = 0.0227$) with a mean decrease of -0.117 points (SE = .2741), while the sham group showed an increase in the LF/HF ratio ($p = 0.0681$) with a mean increase of 0.502 points (SE = .4866) (figure 5c). No statistical differences were found for the power of VLF, LF, or HF.

A two-way ANOVA was conducted that examined the effect of HRV and EDA on arithmetic performance. There was a statistically significant interaction between the effects of HRV and EDA on the math performance, ($F(2, 54) = 4.643, p = .014$) and a (ES $\eta^2 = 0.0871$) indicating that both responses were activated during mathematical performance, and that was associated with and increased attentional state.

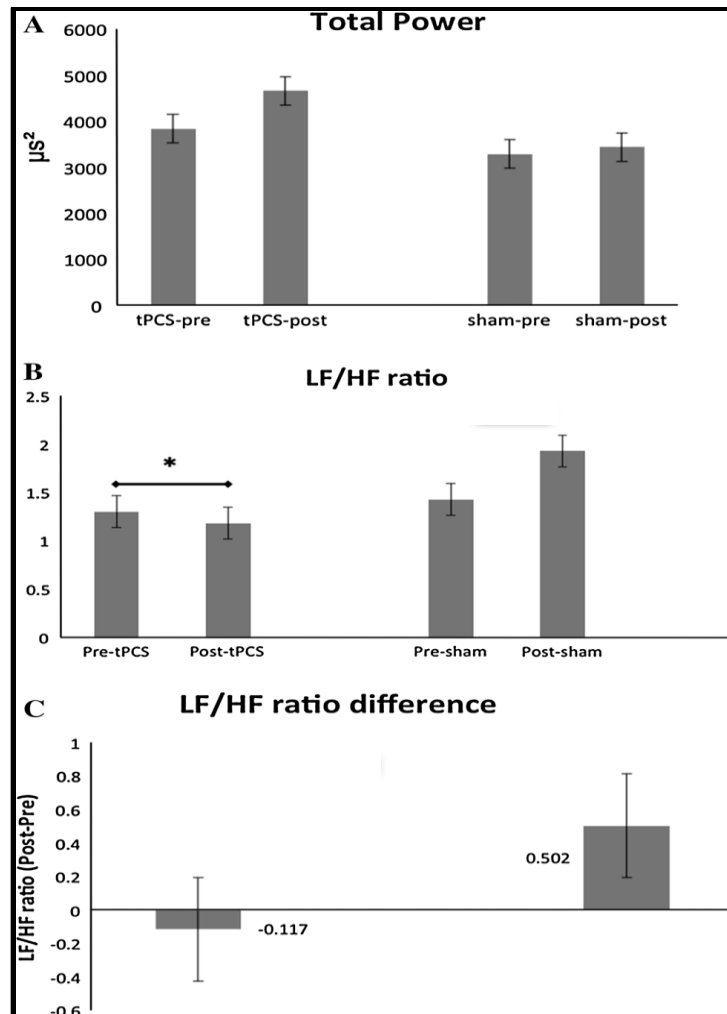


Figure 5.5. Variations in heart variability measurements for active tPCS and sham groups. a) Change in total power. b) Changes in the low frequency/high frequency (LF/HF) ratio. *Asterix* denotes statistical significance. c) Mean decrease difference for sham (-0.117) and increase difference for active tPCS (0.502) in point for LF/HF ratio.

The electrodermal response (EDR) did not presented significant changes in EDR during tPCS stimulation among the three time points ($F(2,56) = 1.779, p = 0.178$). There was no interaction effect of $time \times group$ ($F(2,56) = 0.346, p = 0.709$).

With respect with the validation for blinding , except for one volunteer, all subjects were able to guess correctly their stimulation condition beyond chance, although the level of confidence for their guess measured in a scale from 1 (not confident at all) to 5 (completely confident) was only $3.166 (\pm 1.315)$.

4.4 Experiment 4 (cognitive behavioral and autonomic responses)

For this experiment 30 participants were recruited and all of them completed a single session of stimulation. Participants tolerated well both active and sham tPCS and no substantial adverse effects were reported. Experiment 4 continued exploring the cognitive-behavioral effects of tPCS together with autonomic responses, the main objective was to explore the effects of tPCS on attention and learning, and to observe if the effects were different between the active and the control group, the alternative hypothesis stated that tPCS will modulate attention and learning by improving performance in both task, and that performance will be superior when compared to the control group; the results generated in this experiment are presented below.

For the attention switching task (AST) the mixed model ANOVA showed no significant effects for the switch cost accuracy for stimulation *group* ($F(1,27)=.029$, $p =.867$), *time* ($F(1,27)=2.175$, $p=.152$), or the interaction between *group* and *time* ($F(1,27)=1.109$, $p =.302$). For switch cost RT, however, we found no significant effects of *group* ($F(1,27)=.069$, $p =.795$), but there was a significant effect of *time* ($F(1,27)=15.047$, $p =.001$) (ES $\eta^2 = 0.0965$), and interaction between *group* and *time* ($F(1,27)=19.550$, $p <.001$) (ES $\eta^2 = 0.143$). The post hoc comparisons showed that participants in the active stimulation group were significantly faster ($M=33.300$, $SE=23.690$) than those in the sham group ($M=198,439$, $SE=34.509$) ($p =.010$) (ES $\eta^2 = 0.0841$). Also the active group significantly improved switch cost RT from the pre ($M=198,439$, $SE=34.509$) to the post stimulation period ($M=33.300$, $SE=23.690$) ($p <.001$) (ES $\eta^2 = 0.0967$) (figure 6).

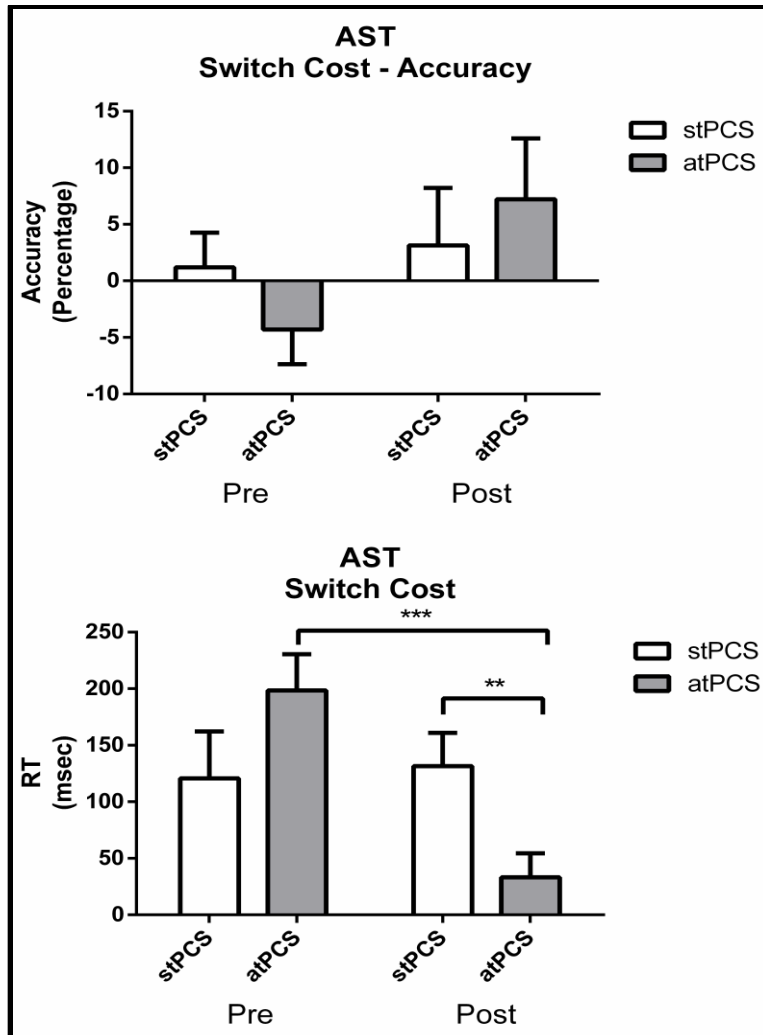


Figure 5. 6. Changes on the attention switching task (AST) for accuracy (switch cost-accuracy) and response time (RT). Active tPCS group improved RT on task when compared to sham, accuracy showed a trend for improved function for the active tPCS group

A significant effect of *time* for PALT ($F(1,27)=24.454, p<.001$) ($ES \eta^2 = 0.0953$) was observed, representing the percentage of recalled words. Participants in both groups were more accurate after tPCS ($M=44.509, SE=2.555$) than in the pre tPCS ($M=36.605, SE=2.955$) ($p <.001$) ($ES \eta^2 = 0.128$). No significant results were found for or *group* ($F(1,27)=.021, p =.886$) or the interaction between *group* and *time* ($F(1,27)=1.198, p=.283$), (figure 7).

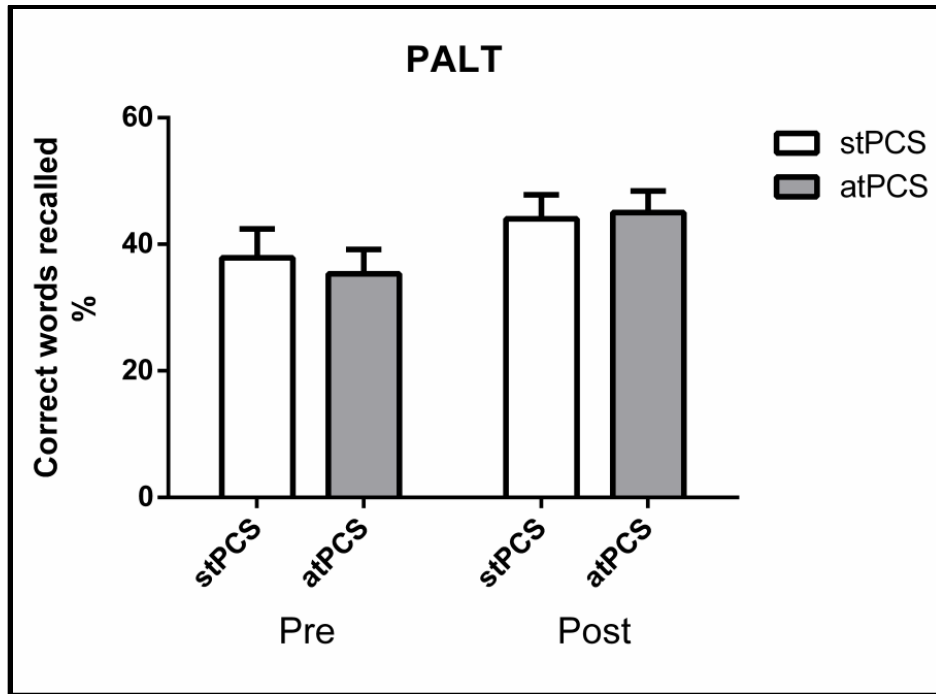


Figure 5. 7. Changes in accuracy in the Paired Associative Learning Task (PALT) for the active and sham tPCS groups. No statistical difference was observed between the groups as both increased their performance in the post assessment.

Physiological responses

ANOVA showed non-significant modulation of HRV total power as compared to sham ($p=0.35$). The LF/HF ratio did not show any difference between groups ($p=0.92$) when compared in the post stimulation period, a minimal decrease of the ratio was observed for the two groups but it was not statistically significant. No significant changes were observed for the rest of the spectral HRV measurements (Figure 8)

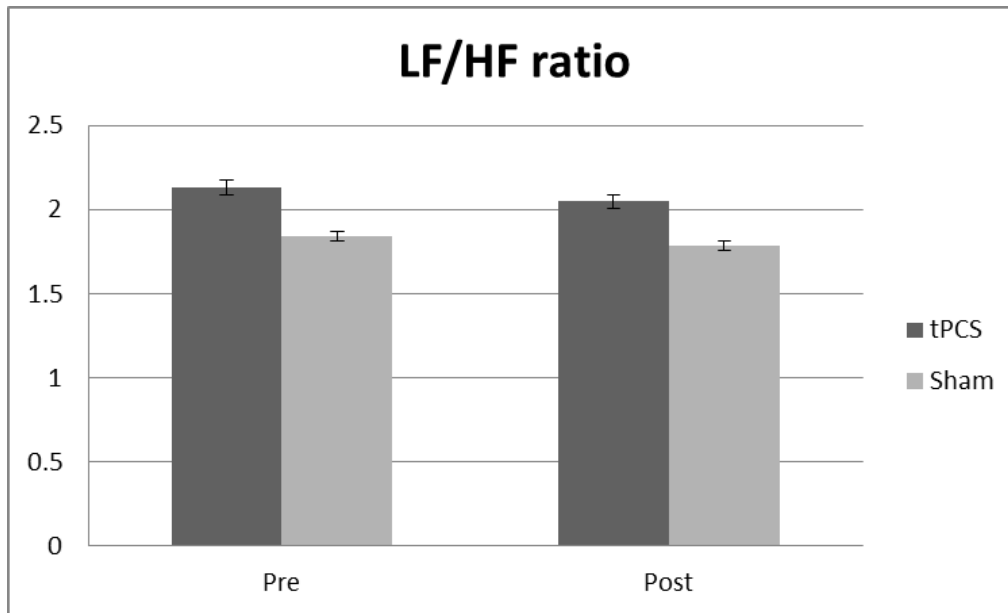


Figure 5. 8. Low frequency, high frequency ratio (LF/HF). Changes in the pre and post evaluation, no statistical difference was observed among groups.

The mixed ANOVA showed no significant effect in EDR responses for *time* for the PALT ($F(1,27)=.029, p =.866$) nor for *group* ($F(1,27)=.290, p =.595$). No significant effects were found for the interaction between *time* and *group* for the PALT ($F(1,27)=3.48, p =.073$) (see figure 9).

The mixed ANOVAs did not show any significant changes in EDR response for the AST task across *time* ($F(1,27)=.041, p =.841$) or *group* ($F(1,27)= .234, p =.633$). There was a significant interaction between *time* and *group* for EDR during the AST ($F(1,28)= 5.107, p =.032$) ($ES \eta^2 = 0.0532$) . Although there was no statistical significance on the post hoc comparison, there was a trend towards increased EDR from pre ($M=5.461;SE= 1.955$) to post in the active tPCS group ($M= 9.165; SE= 2.114$)($p=.077$) (figure 9).

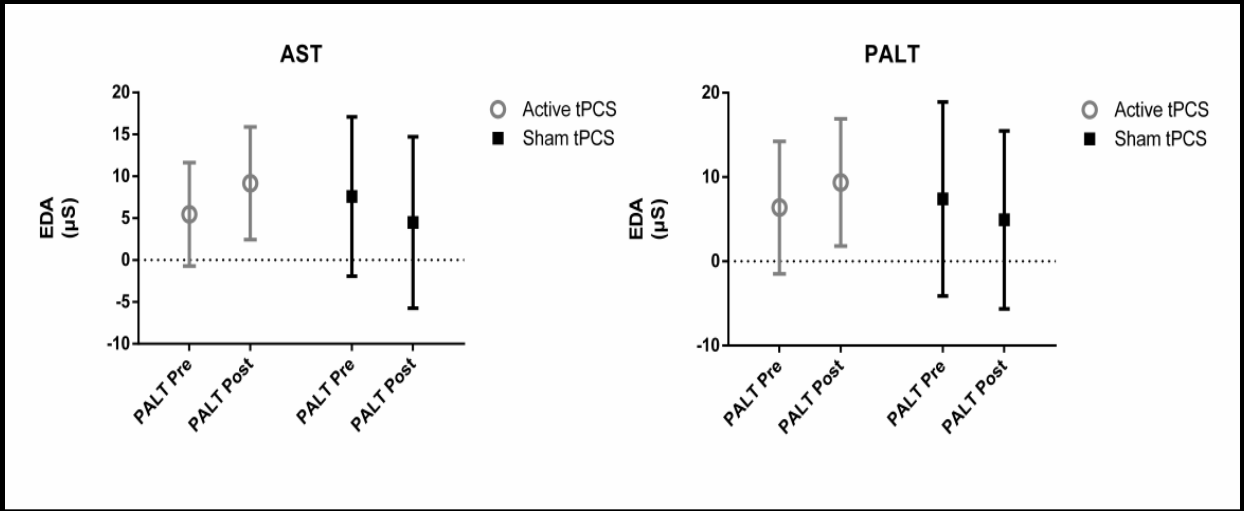


Figure 5. 9. EDR measurements during attention switching task (AST) and the paired associative learning task (PALT), noticed the trend towards EDR increase for the active tPCS group, this increase can be associated with sustained attentional state.

5. DISCUSSION

This work has demonstrated that a simple yet not very well understood NIBS technique is capable to affect electrical brain activity. This is the first time a comprehensive set of experiments were developed with the purpose to shed some light onto the mechanism behind tPCS. Interestingly, the four experiments presented in this work replicated the more important component explored for the purpose of this research, where based on electroencephalographic data, a clear picture started to be recognized as a key unique element of the technique.

Another common denominator in all four experiments was the safety profile tPCS have shown for applications, safety is maybe the most important issue when working with brain stimulation techniques and human subjects, although cranio-electrical stimulation the “umbrella-like term” non-specific technique was approved more than forty years ago, the FDA based its approval in precisely the lack of knowledge about safety, as this might sound backwards, it was shown at that time that CES failed to trigger seizures so this became the standard for safety even though no other components of neurological nor cognitive functioning were explored neither considered.

The organization for this chapter will replicate the format of dividing each discussion per experiment, concluding with a summary and implications for future investigations. The discussion for experiment one will focus on how the best frequency was selected, experiment two denotes the process involved when choosing the current intensity that showed the best effects, and discussion for experiments three and four will describe how the parameters selected from the previous two experiments influence cognitive and behavioral investigations. An addendum will be offered to exemplify two cases where the results from the four experiments were applied in special clinical cases.

5.1 Experiment 1

This experiment was set out to investigate the effects of different tPCS frequencies on cortical activity indexed by high-resolution, high-density array qEEG band power spectrum and inter-hemispheric coherence analysis, this with the intention to understand the mechanisms of this type of noninvasive stimulation technique. It was demonstrated here that active tPCS stimulation with a random frequency ranging between 1 and 5 Hz was capable to significantly increase functional connectivity for the theta and low-alpha frequency bands as compared to sham and active stimulation with either frequencies of 1 or 100 Hz. Post-hoc tests revealed that random frequency at 1 to 5 Hz increased interhemispheric coherence for these frequency bands. There was a small but significant effect on power EEG analysis; but only for the frequency of 1 Hz with a significant decrease in EEG power band. This experiment also confirmed tPCS good tolerability profile as only minor adverse effects were reported and subjects were not able to differentiate accurately between active tPCS from sham tPCS.

The main finding for this experiment was the significant increase in naturally occurring brain oscillations, specifically, theta band interhemispheric coherence observed for the random frequency group compared to sham and either 1 or 100 Hz stimulation. EEG coherence was used as a marker of functional connectivity between different electrode sites, which can then be seen as a surrogate measurement of the potential strength of a given neural network (Boldyreva, Sharova, Zhavoronkova, & Dobrokhotova, 1992). It was clear from the results obtained for this experiment that a random pulsating frequency had a greater impact on EEG measurements, than the applied fixed frequencies which in most EEG measurement produced inhibition of naturally occurring brain oscillations, it is also important to consider the effect size for this result which was robust enough to demonstrate a real difference favoring the active group. These results

offered a particular perspective about differences in pulse frequency design, fixed continuous frequencies are thought to “entrain” ongoing oscillations, while random frequencies or induce “noise” are described to desynchronize the ongoing signal. However, and based on what was observed an induced random frequency was capable to enhance the electrographical properties of connectivity and this happened with a pre-defined signal with random boundaries within the delta-theta range (delta 1 to 4 Hz, theta 4 to 7Hz), interestingly, it was the theta bandwidth who presented a statistically significant modulation, this result can provide evidence of a phenomena where a random artificially generated pulse positively affected a biological signal.

It is hypothesized that neuronal communication is mechanistically subserved by neuronal coherence. As activated neuronal groups oscillate and thereby undergo rhythmic excitability fluctuations that produce temporal windows for trans-neuronal communication (Fries, 2005), EEG coherence represents a state of phase synchronicity which occurs independently of the bandwidth amplitude, this electroencephalographic event needs to happen at certain point in time and between neuronal groups that are becoming more connected as a result of a cognitive processing.

The hypothesis of communication through coherence (CTC) states that coherence is a reflection of synchronization between distant neuronal groups and allows for communication between remote brain areas (Fries, 2005) and spectral coherence can be calculated by special statistical methods commonly used for signal processing, representing the relationship between two signals.

It is thought that theta oscillations coupling, has an important role in information processing and exchange between critical areas involved in memory retrieval and consolidation (Axmacher, Mormann, Fernandez, Elger, & Fell, 2006), as well as for semantic processing . In

general, it has been shown that coherence in the theta band among frontal areas underlies attentionally demanding tasks that are processed at these structures (Polanía, Nitsche, Korman, Batsikadze, & Paulus). Given this context, it is possible to speculate that increases in theta band coherence during rest could reflect a cortical preparedness state, as to enable functional networks to engage in demanding tasks. This hypothesis would be in accordance with studies showing a significant increase in theta coherence between frontal and posterior areas at rest in subjects after mind-body interventions training (Chan, Han, Sze, Wong, & Cheung, 2013) which are believed to elicit a relaxation-like state and internalized attention. Theta band synchronization with gamma or high frequency oscillations (HFOs; >100 Hz) is involved in learning and memory processing in hippocampal areas (Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009), this duality represents the need for a controlled inhibitory tonicity which is mediated by GABAergic activity, this phasic inhibition serves as a “break” for the glutamatergic mediated network activation responsible for the occurrence of HFO’s.

These findings brought the perspective for the development of additional studies: (i) it suggest that tPCS could be further investigated as a neuromodulation technique that can increase functional connectivity in different brain areas; (ii) second, connectivity enhancement depends on the pre-selected induced frequency range used for stimulation. Further mechanistic and modeling methods should be developed as to understand whether this particular effect is associated with brain oscillation entrainment or to noise-induced enhancement of the coherence for the predominant frequency at the time of stimulation delivery, and third, given than these results were observed in neurological healthy volunteers with no alterations in functional connectivity, It is expected the effects of the stimulation are going to be more prominent in pathologic conditions, where connectivity between brain areas may be disrupted or altered. This

assumption – that needs to be tested in further studies – is based on the notion that homeostatic mechanisms including coherence are harder to overcome in neuro-typical subjects. Several studies have shown that cortical oscillatory synchrony can be a robust marker of functionality and organization of cortical networks, providing an interesting window into possible monitoring of therapeutic progress (Dubovik et al., 2012). Some of the cofounders affecting the results of this study are sex, age, and especially mental at the time of the EEG recording, normal healthy subjects who are not drowsy as a result of long testing periods, usually present good EEG reactivity to the testing paradigms (eyes open/eyes close), if these subjects go into a relaxed state, the EEG metrics can be affected, to prevent this mental state of mental relaxation, subjects were engaged to communicate with the experimenter in between periods of EEG recording, also, the EEG technician kept the subjects awake and alert during the process of data acquisition.

5.2 Experiment 2

The obtained results from this study share similar characteristics with those gained in experiment No. 1, with the difference being that intensity of stimulation was the parameter investigated in this trial. Here it was confirmed that a weak electrical pulsed currents can induce visible changes in cortical brain activity as measured by qEEG. Active stimulation using two different stimulation intensities (2 and 1 mA) applied with the same random frequency ranging within the 1–5Hz were able to modulate theta interhemispheric coherence in the fronto-temporal cortical areas as compare to sham. Interhemispheric connectivity for the beta bandwidth high-beta and low-beta sub-bands also showed a statistically significant increase compared with the sham group. The low alpha sub-band presented a tendency to increase its coherence between the chosen electrode pairs (FT9-FT8 and AF7-AF8); the lack of statistical significance can be

attributable to the effect of high variability of alpha among healthy population compared with neurologically injured or chronic patients where alpha variability is decreased (Vespa et al., 2002). Alpha variability might have an effect on coherence measurements because alpha variability denotes how well the thalamocortical generator is functioning as a peacemaker for electrocortical activity at restful state, therefore, during task performance this activity tends to be desynchronized and the oscillation tends to be more “symmetrical” in phase so its coherence increases, in the light of the marginal statistical significance, we can assume our sample of healthy participants presented high alpha variability which somewhat obscure the generation of alpha coherence as the system could not develop this signal symmetry over time. This experiment also confirmed what was previously observed on experiment No. 1, as tolerability and adverse effects shown an acceptable safety profile, so that tPCS can be used for human applications while preserving participant’s safety. Finally, tPCS application can be adequately blinded at least in a parallel trial with participant’s naïve to tPCS.

The observed pattern of interhemispheric coherence with a net increment for the theta and beta bandwidths represents a state of network synchrony among neuronal groups located under the recording electrodes (Ruchkin, 2005), as in the case of experiment No. 1, the effect size associated with these particular changes were strongly in favor of a real effect, rather than just increased or decreased in the variability. This theta–beta coupling can be also associated with the theta-gamma phenomena previously described; it seems that for adequate network synchronization an inhibitory tone must be present, so the faster or higher frequencies can occur without reaching a critical point for hyperexcitability. Theta coherence has been previously involved in working memory processes, which also is accompanied by an increase in the power of theta (Sarnthein, Morel, von Stein, & Jeanmonod, 2005); this phenomena comes in association

with a higher frequency such as beta or gamma oscillations supporting attentional processes within the prefrontal network (Sauseng, Klimesch, Schabus, & Doppelmayr, 2005), as mentioned before low-alpha coherence did not reach significance when compared with the results obtained for theta and beta, it only displayed a tendency to increase, this supports the concept of a bi-modulated network for frequencies that can be considered purely oscillatory in nature, such as in the case of alpha and theta, both bandwidths oscillations are suitable for the functioning of highly organized cognitive processes. Cortical oscillations can be modulated by summation of subthreshold stimuli affecting neuronal firing rate by the ongoing random frequencies. Imaging studies demonstrated BOLD signal modulation by transcranial random noise stimulation, establishing a relationship between the frequency of the signal and the intensity of oxygen metabolism within specific cortical areas (Saiote, Polanía, Rosenberger, Paulus, & Antal, 2013); in this regard dose intensity must also be related with brain oxygen consumption, assuming that increases in power and coherence reflect a higher state of network activity.

Interestingly, these results showed similar qEEG patterns between the 1 and 2mA intensities dosage, indicating that weak current densities within a close range can modulate electrical brain activity with different power attritions; this has been previously demonstrated before in transcranial direct current stimulation studies (Nitsche & Paulus, 2000), and this is in accordance with the idea that membrane polarity shifts can be modified by logarithmic manipulation of the current densities. Accordingly, extremely weak currents like 0.2 mA, did not produce any measurable qEEG changes and behave similar to the sham condition, demonstrating that intensity of the electrical charge was not enough to produce subthreshold facilitation of polarization. In contrast with healthy neuro-typical volunteers, neurologically affected participants may respond different, as in the case of patients affected by a chronic condition,

where maladaptive plasticity phenomena change the oscillatory and connectivity properties for specific networks (Thibaut, Bruno, Ledoux, Demertzi, & Laureys, 2014). Therefore, if a given stimulation dose is able to modulate normal electroencephalographic activity, it might be anticipated that an aberrant circuit can react in a more pronounced manner. These assumptions can be only demonstrated by studies using tPCS in neurologic or psychiatric populations, in pathological conditions it is important to consider the time of disease progression so chronic states might demand stimulation at higher intensities, although this experiment was not design to test this concept, it provides some insight of how the healthy cortex react when exposed to a different stimulation intensities. The confounders affecting these results are the same expressed for experiment No.1, to adequate control for those covariates a larger sample size is needed, due to the exploratory nature of this work, future studies with superiority-like design will help to control this statistical noise.

5.3 Experiment 3

Experiment number 1 and 2 provided direct mechanistic information of how tPCS influence cortical electrical activity. Frequency and current intensity parameters were tested and the results showed that a random frequency with a pre-specified range within physiological EEG parameters had better impact on power and coherence as compared with fixed frequencies or sham stimulation. It was also observed that current intensity has a differential effect based on how strong the stimulation is, accordingly 1mA was capable to elicit measurable changes on the qEEG, but 2mA produced a stronger response when compared with 1 mA, a very low intensity (.1mA), or the sham intervention. Area of stimulation influence was define by the electrographical findings, in both experiments an area contained within the temporal and

prefrontal structures showed the greatest electrographical effect, this corresponds with the area of electrode placement, although the ear clips containing the electrodes were placed extra-cephalically (ear lobes), the current was capable to exert its influence in these structures. The anatomical localization for the stimulation effects follow the principles of current source and topographical positioning and this is true for most of the noninvasive neuromodulatory techniques (Marom Bikson, Datta, Rahman, & Scaturro, 2010) as the position of stimulating electrodes governs current flow through the body, and hence the distribution of induced electric fields in the brain. These induced cortical currents/electric fields modulate neuronal excitability for DC stimulation and, in turn, determine behavioral and clinical outcomes (M. Bikson et al., 2008).

After experiment 1 and 2 deliver a clearer picture of tPCS parameters, the next reasonable step was to test those parameters for the modulation of cognitive behavioral functioning, thus experiment 3 was developed for that specific purpose.

In order to test whether the modulation of such oscillations has an effect on specific cognitive and behavioral tests, The experiment was designed involving tasks with a functional component linked to the anatomical structures contained within the regions directly influenced by the stimulation, as observed in neurophysiological and modeling studies. The results from this experiment showed that tPCS has a specific, and marginally significant effect in a complex arithmetic task in healthy individuals. In fact, there were no significant effects in the Stroop and Bart tasks. Although these findings do not fully confirm the main hypothesis, they present critical insights for the future development of tPCS as neuromodulatory technique and for better understanding of the main determinants for response. Given the marginally significant behavioral effects obtained in this study and the investigation of other parameters (such as

intensity), it is likely that a larger number of sessions may increase the effect size of tPCS. It is well known the additive effects of multiple stimulation sessions in cognitive performance (Reis et al.). Another possible reason for these results may be the population being investigated: healthy subjects. Given the likely effect of tPCS in strengthening pre-existing neural connections and thus inducing cognitive enhancements, individuals with no major impairment in connectivity may have modest or no cognitive effects at all by only having tPCS. It can be inferred that a healthy neuro-typical participant has no much capability for improvement as the system it is already working at its optimal or near optimal state.

Though effects were modest for the complex arithmetic tasks, a hypothesis can be presented to explain such effect. The parietal cortex, specifically the left angular gyrus, has been involved in arithmetic fact retrieval processing for mental calculations, while a broader area extended over the fronto-parieto-occipital network including the basal ganglia seems to be involved in procedural mental operations (Grabner, et al., 2009). Hence, performance in a task with a procedural component requiring approximations for problem-solving can be enhanced by tPCS modulation of the engaged cortical areas by facilitation of connectivity among neuronal groups involved in arithmetic processing and decision making as domain-specific attentional modulation occurs in the temporo-prefrontal cortex, rather than performance in arithmetic activities requiring pure parietal activation for exact problem resolution, which involves the retrieval component. As procedural mental processes require an extensive connectivity network effort, the induction of theta coherence mediated by a random frequency can somewhat facilitate the behavioral strategies used for problem-solving; for instance, mental calculation has been proven to increase coherence of the theta frequency band in frontotemporal areas (Nunez, Wingeier, & Silberstein, 2001) by selective activation. Interestingly, the structures involved in

working memory and attention (prefrontal cortex and medial temporal lobe) are located under the area of tPCS influence. Moreover, the generation of gamma oscillations is associated with arithmetical processing while theta is being coupled to gamma as inhibitory force to the glutamatergic gamma tone. It is important to note that overall and without statistical differences between the groups participants performed faster during the congruent stimuli on the Stroop test than during trials with non-congruent stimuli, although this can be explained by a learning effect, this phenomena could facilitate the effects of the stimulation by a sort of bottom-up modulation in the active tPCS exposed group.

No significant differences in the pre- and post- measurements were observed on the BART task indicating a different circuitry involvement in the processing of risk-taking behavior which is anatomically located in the mesolimbic-frontal regions. Nevertheless, there was a clear tendency for the tPCS active group to perform toward a conservative behavior when compared to the sham group, indicating a phenomenon of symmetry establishment between the left and right hemisphere as a consequence of the induced theta inter-hemispheric coherence promoted by the random pulsed frequency. It has been mentioned that increased theta power in frontal regions (Schutter, Leitner, Kenemans, & Honk, 2006) and right to left theta asymmetry in the prefrontal area (Studer, Pedroni, & Rieskamp, 2013) are findings related to increased risk-taking behaviors. Taking into account the lack of effect when the analysis was done considering the type of intervention on the Stroop task, we can assume tPCS did not facilitate cognitive flexibility, nor the ability to deal with increased cognitive load, as selective and divided attention are processed in the realms of the cingulate and prefrontal network; therefore, the effects of tPCS in the arithmetic task can be seen specific for the network involved in problem-solving.

Experiment 3 not only explored the effects of tPCS on cognition, two markers of the autonomic nervous system (ANS) were also investigated with the purpose to observe modulatory effects on the parasympathetic system. The ANS is in charge of “self-regulation” and is distributed centrally and to the peripheral tissues and organs. The controlling centers are located in the hypothalamus and the brainstem. From these centers neurons are projected out of the CNS to synapse on multipolar neurons in the autonomic ganglia. The autonomic system is further divided in two components the sympathetic and parasympathetic systems. The sympathetic system acts in “sympathy” with the emotions, especially in association with rage or fear, it prepares the body for the “flight or fight” response which is characterized by increased hearth rate, pupillary dilation, and skin sweats. The parasympathetic system counterbalance the effects from the sympathetic system by reversing the physiological response: slowdown of the hearth rate, increased intestinal activity, and salivary secretion (Mtui, Gruener, & FitzGerald, 2011).

Heart rate variability refers to subtle beat-by-beat variation in the heart rhythm. In healthy subjects, each heart beat is initiated by the sinoatrial node located in the posterior wall of the right atrium. The cardiac cells located in this area exhibit what is referred to as a leaky conductance across their membranes which results in a regular and uniform discharge of action potentials that cause the heart to contract at a constant frequency. Ordinarily, however, many factors constantly modulate the autorhythmicity of sinoatrial firing rate, as in the case of physical activity or cognitive load. This is achieved by way of the autonomic nervous system's two opposing forces: the sympathetic and the parasympathetic (vagal) tonicity. By examining heart beat-to-beat variation one therefore effectively gains insight into autonomic nervous system tone when exposed to a different tasks or stimuli. It is well know that anxiety and decision making are associated with increased HRV, specifically decreased high-frequency HRV (HF-HRV) and

increased low frequency-high frequency ratio (LF/HF) are markers of increased sympathetic tone (Ramirez, Ortega, & Reyes Del Paso, 2015). The obtained results in HRV revealed an influence of tPCS over the central autonomic network (CAN). The active stimulation group developed an increase in HRV total power accompanied by a decrease in the LF/HF ratio, reflecting a state of sympathovagal balance. It is important to notice that one of the first signs of stress is tachycardia, and this usually precedes a marked reduction in the total power. HRV measurements were recorded throughout the experiment, and the post-recordings were obtained immediately after the cognitive tasks were completed; thus, individuals who received tPCS displayed better sympathetic control after exposure to stressful conditions. Furthermore, the active tPCS group developed a decrease in the LF/HF ratio, whereas the sham group exhibited an increment of the same measurement, indicating that tPCS facilitated sympathetic modulation. Although no significant differences were found for LF and HF power spectrum, which are thought to represent sympathovagal control, changes in LF/HF balance and the HRV total power may reflect reciprocal changes in sympathetic and parasympathetic activity in this realm (Reyes del Paso, Langewitz, Mulder, Roon, & Duschek, 2013). However, the shifts from parasympathetic influence to sympathetic activation to the sympathovagal balance are known to be confounded by the prevailing heart rate and the mechanical effects of respiration; therefore, the present results should be seen as preliminary. Although EDR did not significantly change through the tasks, there is a trend in the tPCS group to increase its response as compared to the sham group, indicating perhaps a sustained state of attention. Therefore, additional studies are needed to fully understand the effects of tPCS have over the CAN. research has showed that individuals with higher resting HRV (such as total power) exhibit faster response times and better accuracy on executive cognitive tasks (Hansen, Johnsen, & Thayer, 2003). Changes in vigilant versus resting

state can also have a major impact on sympathovagal balance and vice versa. These variations in LF/HF balance and the HRV total power may reflect reciprocal changes in sympathetic and parasympathetic activity over cardiac control. In fact if, tPCS may have a direct vagal effect (independent on the brain modulation), due to the stimulation of the Arnold's nerve located near the earlobe and the tragus of the ear canal, this nerve is a branch of the vagus nerve so the tPCS electrodes might modulate parasympathetic activity by peripheral means.

Experiment No. 3 is the first of its kind to demonstrate tPCS properties when applied under controlled conditions. This experiment provided additional data supporting the modulatory effects of tPCS. Although the behavioral effects were modest and the physiological evidence pointed towards some interesting concepts, they are helpful as represent an antecedent for the design of further studies. Additional experiments are needed in order to elucidate the mechanistic attributes of weak pulsed currents and its interactions with endogenously generated oscillations during cognitive processing.

5.4 Experiment 4

This study revealed that active tPCS significantly improved response time in the AST compared to the sham condition, so that participants receiving active tPCS significantly exhibit decreased switching cost between repeat and switch trials. No differences were observed in response accuracy for switch trials. Active tPCS had no significant effects on accuracy for the PALT. For this experiment, no significant changes were recorded in physiological parameters such as HRV and EDR, for either the active or sham tPCS groups.

The capability to switch between different cognitive processes, such as shifting attention from one task to another, or the ability to engage in different activities at the same time, is one of

the most distinctive capabilities of human beings that allow us to successfully interact with our environment (Wylie, Javitt, & Foxe, 2003a, 2003b). This ability represents a key mechanism in behavioral flexibility and is thought to rely mainly on executive control processes and attention (Rubinstein, Meyer, & Evans, 2001), requiring the complex engagement of multiple, interconnected brain circuits, including mainly the parietal and frontal cortices (Calzavara, Maily, & Haber, 2007; Haber, Kim, Maily, & Calzavara, 2006), as well as input coming from the basal ganglia (Haber & Calzavara, 2009). Our finding of increased response speed for the AST suggests that tPCS is able to modulate the complex interaction of the areas involved in the cognitive processing required for responding to a switch between rules.

Although no changes were found in performance enhancement, the improvement in response time for the AST may indicate that tPCS increases the efficiency of the neural connections of the underlying related neural network. The improvement in neural efficiency is not a novel concept, as it has already been shown that repeated performance is associated with response time and cortical activation decrease (Garavan, Kelley, Rosen, Rao, & Stein, 2000; Olesen, Westerberg, & Klingberg, 2004). As tPCS is a type of random noise stimulation, the tPCS induced effects could rely on a mechanism called stochastic biological facilitation, where noise tends to amplify and make more coherent a weak signal (Srinivasan, Winter, Ding, & Nunez, 2007). Therefore, facilitation can be controlled by the predefined boundaries of the selected ranging frequency.

Although this study did not compare the effects of tPCS and tDCS on task performance, it is relevant for our discussion to explain some of the differences between these two non-invasive brain stimulation techniques given that there is an increased interest in understanding specific effects of different techniques of non-invasive brain stimulation. tDCS is able to

modulate spontaneous cortical activity and excitability in a polarity-dependent way, leading to secondary changes in synaptic strengthening (L. J. Bindman, O. C. Lippold, & J. W. Redfearn, 1964; Purpura & McMurtry, 1965). The synaptic changes induced by tDCS make this technique a desirable option for enhancing learning, as it promotes LTP/LTD process making the synapsis stronger of weaker depending of the stimulation polarity, which in turn will drive the learning process. In fact, several studies have shown that tDCS induces performance enhancement, rather than an improvement in efficiency, in cognitive processes related to attention and working memory (Bolognini, Fregni, Casati, Olgiati, & Vallar, 2010; Carvalho et al., 2015; Fregni, et al., 2005; Zaehle, Sandmann, Thorne, Jancke, & Herrmann, 2011) as well as task switching ability (Leite, Carvalho, Fregni, Boggio, & Goncalves, 2013; Leite, Carvalho, Fregni, & Goncalves, 2011). These studies suggest polarity and task specific effects, especially on complex versions of the task where increased accuracy is associated with decreased speed (Leite, et al., 2013), these results exposed a modulation of the system to what we can conceived as an optimization of function which might not be dependent on energy expenditure, but rather in synaptic efficiency.

Experiment number three suggested a different effect of tPCS on cognitive processing. For instance, tPCS has already been shown to moderately improve performance on two and three digits arithmetic operations, which has been thought to be the result of wider network modulation over the fronto-parieto-occipital networks, specifically for a task which involves a more procedural component rather than an calculation retrieval present on the one digit mental operations (Morales-Quezada et al., 2015).

Therefore, it is possible that the changes observed in RT for the AST with no parallel improvement in accuracy, or general performance, represent the bottom-up (i.e. subcortical-cortical) facilitatory effects of tPCS on functional connectivity enhancement of pre-existing

neural networks. Although this is just a hypothesis, there is some evidence to support this claim. First of all, performance on the AST task is dependent on cortico-striato-thalamo-cortical (CSTC) loops (Sylvester et al., 2003). Consequently, the required modulation of neural activity in order to impact behavior can occur at cortical, subcortical or at both levels. Previous tPCS computational simulations suggest that the conventional ear clip montage of tPCS is associated with a current distribution located in cortical temporal regions, but also with a diffuse activation over the thalamus, insula and cingulate, among others (A. Datta, et al., 2013). Thus, it is possible that the results presented in this experiment can be explained by a neuromodulatory influence over subcortical structures, which in turn are modulated by a bottom up control mechanism would then have relayed the information to the cortical integrators, thus resulting on improved RT performance.

The fact that the improvement was only for speed and not accuracy further strengthens the hypothesis. Witt and Stevens (2013) using a paced tapping task in the absence of cues, showed that subjects who performed better, relied more on top-down control of motor and sensory regions, while worse performers exhibited a sensory driven (i.e. bottom-up) approach.

This study suggests that task accuracy could be dependent on top down control, with the bottom up control resulting in a deleterious effect. The lack of significant results on accuracy on both AST and PALT could be possibly explained with the fact that higher efficacy requires engagement of more cortically based neural circuits; which is not a result of tPCS effects. In fact, the results point to tPCS leading to a more pronounced effect on the processing of tasks that require a wider and more engaged activation of pre-existing neural circuits, possibly by a bottom up mechanism. Such differences should be further studied both in healthy subjects and in

populations with neurologic or psychiatric conditions, as to identify possible response determinants and associations.

This tPCS putative mechanism of action is also supported by previous experimental work testing the neural effects of tPCS (Castillo Saavedra et al., 2014; Morales-Quezada, Saavedra, Rozisky, Hadlington, & Fregni, 2014). Previous experiments showed that tPCS modifies functional connectivity between pairs of electrodes corresponding to temporal-prefrontal areas, as indexed by an increase in interhemispheric theta and beta coherence measured by quantitative EEG. This can also be a result of subcortical-cortical modulation (Malekmohammadi, Elias, & Pouratian, 2015). For instance, a pacemaker system constituted by thalamic neurons intrinsic oscillatory firing pattern has already been shown (Blethyn, Hughes, & Crunelli, 2008; Hindriks & van Putten, 2013). Such pacemaker system will then be able to generate and synchronize oscillatory activity through thalamocortical circuitries (Drover, Schiff, & Victor, 2010; Hindriks & van Putten, 2013); and by a thalamocortical phase-amplitude coupling (PAC) mechanism, lower phase thalamic frequencies can modulate the activity of equal or higher phase cortical frequencies (Malekmohammadi, et al., 2015) which are associated with task performance. Interestingly enough, this importance of the thalamus on selective attention by regulating thalamocortical information has already been extensively reported (Crick, 1984; Guillery, Feig, & Lozsadi, 1998; Yingling & Skinner, 1975).

The repeated coupling could have then increased performance speed by strengthening of synaptic connections, which has been discussed for other tasks that require rapid reaction time (Gold & Shadlen, 2002; Lo & Wang, 2006). This hypothesis states that increasing neural connections between recruited circuits leads to a decrease in the time needed to respond to a stimulus, such that incoming information is quickly processed by subcortical centers and

transmitted to the cortex so that a response can be elicited. By strengthening the connections between subcortical and cortical networks, the threshold required by the cortex to elicit a response decreases, therefore enhancing response time (Bogacz, Wagenmakers, Forstmann, & Nieuwenhuis, 2010). This theory also emphasizes on the significant trade-off between speed and accuracy, such that emphasis on speed improvement can lead to a decrease in accuracy, which is associated to the findings with no changes in accuracy performance. Therefore, the relationship between these two factors can be described as an inverted-U-shaped, in which accuracy is affected when speed increases beyond a critical point (Gold & Shadlen, 2002). Achieving a balance between both requires multiple trials and is usually associated with positive reinforcement (Furman & Wang, 2008; Gold & Shadlen, 2002), which leads us to believe that in this case the trade-off between speed and accuracy could be optimally achieved after repeated sessions of stimulation paired to a cortically demanding task.

Although physiological measurements show no significant changes, the trend for an increase in EDA during active tPCS is consistent with the effects promoted by cognitive testing, as both of them (AST and PALT) required a high level of sustained attention. Future studies need to control for some important covariates including individual's physical characteristics such as gender, age, and physical condition as to decrease variability of response; and thus to understand how tPCS modulates autonomic responses.

This experiment provides further data supporting the behavioral effects of tPCS and support future investigation exploring the specific effects of tPCS especially among other NIBS techniques, or by combining tPCS with these other NIBS techniques.

6. LIMITATIONS

The development of these four experiments provided valuable information in regards tPCS effects on healthy subjects. This assumption – that needs to be tested in further studies – is based on the notion that homeostatic mechanisms modulating EEG metrics are more difficult to overcome in healthy subjects. Several studies have shown that cortical oscillatory synchrony can be a robust marker of functionality and organization of cortical networks, providing an interesting window into possible monitoring of therapeutic progress, thus, generalizations to clinical populations are difficult to apply. However, if the observed changes occurred in a “healthy” or normal oscillatory brain, it seems plausible to believe a deeper effect will occur in a system with electrically faulty oscillations. Another issue to consider is the fact that a big number of participants were younger than 40 years, in the aggregate of population, that issue will skewed the curve towards the left, however, in every experiment the standard deviation for age was within acceptable values of normality, the inclusion of a wider age group would alleviate somehow this statistical concern, unfortunately the demographics for study participation in a city like Boston, seems to favor the young students population. Another possible option in order to understand the effect of age in our results, is to perform a stratified analysis with the total cumulative sample size, although this was a difficult task during the analysis process due to the nature of how the experiments were organized, this question deserves an answer that can be obtained through a secondary analysis of the whole dataset. Another statistical option for the secondary analysis is to perform an analysis of covariance (ANCOVA) by expanding the ANOVA functionality (in a linear regression fashion) and controlling for covariates such as age. EEG measurements change over the lifespan so it will be important to adjust per age group in future studies were EEG plays an important role as an outcome.

Gender could be another factor influencing our results, especially for physiological measurements. It is well known that menstrual cycle has an effect on HRV measurements, or the instability of the EDR due stimulants consumption (e.g. coffee, energy drinks, or physical activity), in order to control for sex or any other covariate, while having a minimum sample size with enough power to detect such changes, it would require the development of an another experiment including just females, or stratifying per specific variable, such a design will require for a bigger study including both sex. Nevertheless, as the experiments designs were exploratory in nature, these sorts of statistical questions can be addressed in future studies with different alternative hypothesis to be tested.

Another problem that might confound the results is the learning effect to a standardized task when subjects are exposed to a pre and post evaluation, although some tasks are prone to be sensitive to this form of learned effect as in the case of the word memory task or the Stroop task, another assessments are less impacted by the “learned task” phenomena, for instance, the arithmetic task, BART, and AST all have specific psychometric properties that prevent the learning to occur due to the intrinsic nature of the task. The medium to the complex level of performance for the arithmetic will require explicit mathematical calculations which will demand previous knowledge of basic math performance based on consolidated learning, but the numeric variables contained in the problem represents a *de novo* challenge so the learned task phenomena is not well kept in between testing trials. Both the AST and BART embrace complex neurocognitive processing that demands high levels of mental load, among the features preventing the learned task effect is the randomization of the stimuli itself and the context of the response, e. g. selection of the correct answer to the presented cue for the AST, or the drive to gamble and earn money in the BART. Mathematically, the analyses accounted for the learned

task effect by correcting for baseline, so the true mean difference between groups is presented under this correction.

To obtain EEG metrics some mathematical assumptions are made, specifically about brain dynamics. The fast Fourier transformation or FFT calculation is a linear method that assumes the system behaves in a static way over a time series, while these assumptions does not reflect real brain functioning, it allows for gathering objective values that can be used for statistical comparison. Other advanced and sophisticated methods can be used to transform the raw EEG data, for instance, independent component analysis (ICA) or wavelets transform (WT) to mention some have better mathematical resolution, but the tradeoff is an increase on computational power which makes the analysis longer to obtain, the equipment limitation in our center prevented us to use any of the methods described above. By applying FFT as EEG transformation tool, and by observing the obtained behavioral data, we then can accept the overall validity of the results based on previous knowledge that supports and provides physiological ground to what we observed.

7. CONCLUSIONS

The work presented in this thesis epitomizes the experiments initially developed with the intention to provide a better understanding of tPCS as NIBS technique. The first results pointed out to what the author was more or less expecting in terms of mechanistic properties of this stimulation, however, as the investigation moved towards more complex designs a different picture started to be drawn. The first main result showed the effects of a random frequency had on EEG recordings. Based on previous research exploring alternating currents, a presence of brain oscillatory rhythms entrainment was expected, nevertheless, the random frequency applied

during the stimulation revealed that randomness not only increased the recorded signal of “near-by” or neighborhood frequencies, but also facilitated their signaling for connectivity, contrary to alternating fixed frequencies, where a given frequency constantly alternating at the same rate will promote the oscillation of the system ensuing the same rate (*i.e.* alpha entrainment at 10Hz).

Then, a second experiment demonstrated that 10 and 20 minutes of stimulation will have a positive neuromodulatory effect as recorded by qEEG, but not 30 minutes. These results were online with previous studies using other forms of NIBS, and it also put on evidence the role of physiological phenomena, meaning that 10 and 20 minutes will sustained an influence over the oscillations, but once the physiological threshold is crossed, the system will not be longer be affected by the stimulation. Important to notice in the second experiment was the replication of the EEG results obtained in experiment number one. Experiments three and four not only showed and replicated the electrographical modulations observed in experiments one and two, but also demonstrated that tPCS had an effect in cognitive tasks involving the cortical territory exposed to the stimulation electrical field, moreover, the results exposed tPCS as an enhancer of optimization rather than performance by accuracy as RT and broad networks were affected for attention switching and arithmetic functioning respectively. Another important issue to consider was safety and tolerability as no main adverse events were reported other than tingling and non-specific sensory symptoms. Given the fact that all of the participants were exposed just to a single session of tPCS, it remains open the question of what would happen if subjects are exposed to repetitive stimulation? Also important to consider is what would happen if the stimulation is associated with online cognitive training, so by activation, we promote functional targeting of cortical structures. All of these points are valid questions for further investigations

using tPCS as NIBS intervention for both cognitive performance in healthy subjects and for cognitive remediation in patients with neurological disorders.

It is safe to say that tPCS modulates electrical cortical activity, however, the mechanisms responsible for such modulations still elusive and not completely understand. An attractive hypothesis involves a phenomenon that has been known in the field of signal detection and signal analysis. Stochastic resonance, where a dynamical system presents itself with periodic oscillations can be altered by the motion in frequencies of an external harmonic generator. When the generator is randomly oscillating (also called perturbation) can promote a resonance in the signal which can be detected by enhancing its power or by facilitating its coherence (Benzi, Sutera, & Vulpiani, 1981). The CNS has oscillatory properties, starting from the single cell unit all the way to organized neural networks; all of these components have shown to be affected by perturbations when introduced into their own spectrum, in this sense the neural systems are natural displays of stochastic resonance (Rudolph & Destexhe, 2001). When the EEG data was analyzed, it came with a distinctive pattern in all four experiments; 1) it increase the power of the signal, 2) affected frequencies located in the vicinity of the analyzed spectrum, and 3) increased the coherence between recording electrodes. These properties support the concept of tPCS as facilitator of connectivity within the network exposed to the stimulation.

So far the four experiments were carried out in neuro typical participants, and all of them only receive a single session of stimulation, therefore, still unknown the accumulative effects of tPCS, or if this technique has some effects on pathological conditions.

Future investigations are needed in order to gain a better understanding of the technique, and additional investigations exploring tPCS for cognitive performance are required before we consider its applications in neuropsychiatric disorders. However, these are interesting times for

the field of NIBS. Current models of treatment involving pharmacological interventions remained not satisfactory in terms of efficacy and remediation of neurocognitive deficits, therefore, tPCS as other forms of brain stimulation will start being considered for treatment and rehabilitation, this in turn, will change the way health professionals conceive neuromodulation as an option to provide an adequate integrative care.

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APPENDICES

Institutional Review Board

Tel: 617.952.6182



SPAULDING[™]
REHABILITATION NETWORK

Spaulding Rehabilitation Network
Research Institute

79/96 Thirteenth Street
Charlestown Navy Yard
Charlestown, MA 02129

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Initial Review: Notification of IRB Approval/Activation Protocol #: 2013P001065/SRH

Date: August 23, 2013

To: Felipe Fregni, MD, Ph.D
SRH
SRH Dept of PM and R

From: Spaulding Rehabilitation Network Research Institute
79/96 Thirteenth Street
Charlestown Navy Yard
Charlestown, MA 02129

Title of Protocol:	Transcranial pulsed current stimulation (tPCS) for cognitive modulation: assessment of optimal parameters of stimulation and investigation of mechanisms of action.
Version Date:	7/8/2013
Sponsor/Funding Support:	BrainGear
Study Population:	Adults
Consent/Authorization:	Required
Documentation of Consent:	Written
Informed Consent From:	Adult Subject
Informed Consent By:	Non-Physician Investigator
IRB Review Type:	Full
Risk:	More than Minimal Risk
IRB Approval Date:	6/10/2013
Approval Activation Date:	8/23/2013
IRB Expiration Date:	8/21/2014

This project has been reviewed by SRH IRB . During the review of this project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for obtaining and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

Please note that if an IRB member had a conflict of interest with regard to the review of this project, consistent with IRB policies and procedures, the member was required to leave the room during the discussion and vote on this project except to provide information requested by the IRB.

Official Version Generated from the Partners Human Research Committee Database
08/23/2013 10:47 AM



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Approved Documents

Protocol Summary (version date: 7/08/13)

Detailed Protocol (version date: 7/08/13)

Consent Form- Healthy Adults Experiment 3 (version date: 07/08/13)

Consent Form- Healthy Adults Experiment 2 (version date: 07/08/13)

Consent Form- Healthy Adults Experiment 1 (version date: 07/08/13)

Consent Form- Healthy Adults Experiment 4 (version date: 08/07/13)

Consent Form-Healthy Adults Experiment 4 (version date: 08/07/13)

Advertisement

Flyers (2)

Telephone Script

tPCS Screening

Pre-Screening Form

Demographic Data

Blinding Questionnaire

Mini Mental Status

tPCS Side Effects Questionnaire

As Principal Investigator, you are responsible for ensuring that this project is conducted in compliance with all applicable federal, state and local laws and regulations, institutional policies, and requirements of the IRB, which include, but are not limited to, the following:

1. Submission of any and all proposed changes to this project (e.g., protocol, recruitment materials, consent form, status of the study, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB as an unanticipated problem.
2. Submission of continuing review submissions for re-approval of the project prior to expiration of IRB approval and a final continuing review submission when the project has been completed.
3. Submission of any and all unanticipated problems, including adverse event(s) in accordance with the IRB's policy on reporting unanticipated problems including adverse events.
4. Obtaining informed consent from subjects or their legally authorized representative prior to initiation of research procedures when and as required by the IRB and, when applicable, documenting informed consent using the current IRB approved consent form(s).
5. Informing all investigators and study staff listed on the project of changes and unanticipated problems, including adverse events, involving risks to subjects or others.
6. When investigator financial disclosure forms are required, submitting updated financial disclosure forms for yourself and for informing all site responsible investigators, co-investigators and any other members of the study staff identified by you as being responsible for the design, conduct, or reporting of this research study of their obligation to submit updated Investigator Financial Disclosure Forms for this protocol to the IRB if (a) they have acquired new financial interests related to the study and/or (b) any of their previously reported financial interests related to the study have changed.

The IRB has the authority to terminate projects that are not in compliance with these requirements.

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Questions related to this project may be directed to Catherine E
Sutherland, CSUTHERLAND1@PARTNERS.ORG, 617-952-6182.

CC: Kayleen Marie Weaver, BA, SRH - SRH Dept of PM and R, Research Coordinator/Manager

Subject Initials: _____ IRB Protocol # _____

Date: ___/___/___ Time of Study _____

Investigator Name: _____

Short Study Title: _____

tPCS Screening Questionnaire

Have you ever:

Had tPCS before? _____ Yes _____ No

Had an adverse reaction to tPCS? _____ Yes _____ No

Had a seizure? _____ Yes _____ No

Had an unexplained loss of consciousness? _____ Yes _____ No

Had a stroke? _____ Yes _____ No

Had a serious head injury? Yes No

Had a surgery to your head? Yes No

Had any brain related, neurological illnesses? Yes No

Had any illness that may have caused brain injury? Yes No

Do you suffer from frequent or severe headaches? Yes No

Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding? Yes No

Do you have any implanted medical devices such as cardiac pacemakers or medical pumps?
 Yes No

Are you taking any medications? Yes No

tPCS Side Effects Questionnaire – Session _____

Patient Initials:

Date:

<i>Do you experience any of the following symptoms or side effects?</i>	<i>Enter a value (1-4) in the space below.</i> <i>1-Absent</i> <i>2-Mild</i> <i>3-Moderate</i> <i>4-Severe</i>	<i>If present: Is this related to tPCS?</i> <i>1-None</i> <i>2-Remote</i> <i>3-Possible</i> <i>4-Probable</i> <i>5-Definite</i>	<i>Notes</i>
Headache			
Neck Pain			
Pain in area of electrodes?			
Burns in area of electrodes?			
Tingling			
Skin Redness			
Sleepiness			
Trouble Concentrating			
Acute Mood Change			
Dizziness			
Other (specify):			

Stimulation Confidence Rating

Answer the questions to the best of your ability:

Did you receive:

Sham Stimulation

Active Stimulation

Rate how confident you feel in your answer (please check one):

1 Not confident at all

2

3 Somewhat Confident

4

5 Completely confident