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Transcranial direct current stimulation effects on cortical excitability and learning during a dorsiflexion motor task.

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CONTENTS

Abstract

Transcranial direct current stimulation (tDCS) is a method that could induce changes on the corticospinal excitability and enhanced motor learning. Nevertheless, research on the topic still ongoing due to the great variability of the corticospinal response and different methodologies that has been used with this device. Moreover, there is not much evidence on how it could affect to the lower limbs. Therefore, the aim of this study is to see what are the effects of a long-term exposure to tDCS and if they are maintained after its exposure. Thirteen right-footed healthy participants were recruited that were double blind and randomly assigned to different groups SHAM or STIM condition. They performed a motor task during 5 days and it was assessed 8 days after the last practice. Corticospinal measurements I/O curve, SICI and silent period were assessed before and after day 1,5 and retention day. Motor task consisted in following a sinusoidal curve displayed on a screen with an isometric force applied through a dorsiflexion of the ankle muscles. Result were no significant improvement from SHAM group from pre-to-post measurements on day 1. Non-significant results were found in the rest of the conditions, motor task error, Input/output curve, SICI or cortical Silent Period due to the dispersion of the data. Therefore, it cannot be concluded that tDCS will enhance the motor learning. However, it does increase the variability of the corticospinal excitability after its use.

1. Introduction

The motor cortex is an area of the brain, which is related to voluntary movements. The primary motor cortex is located within the motor cortex. It has direct connection with the spinal cord and the motor neurons (Figure 1) (Enoka 2008, pp.249-300). Therefore, an increase on synaptic connection within the cortical track will increase the ability to perform a motor skill (Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett 2001).

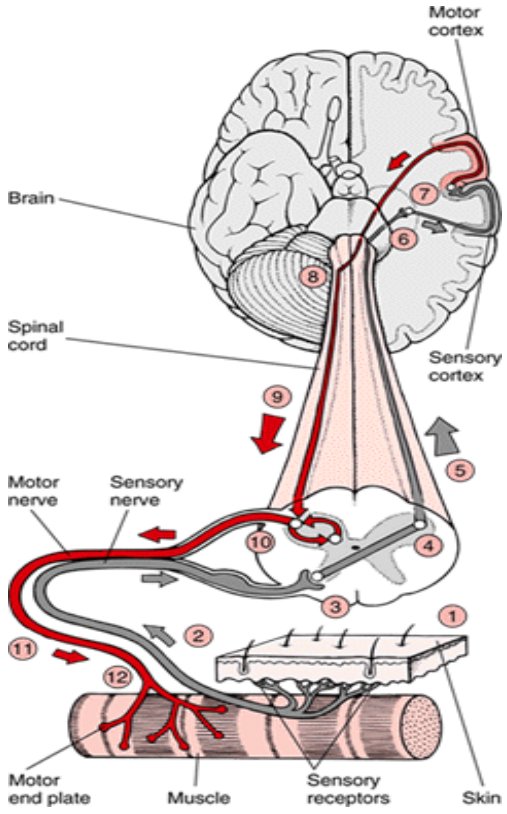

FIGURE 1. Central and peripheral nervous system. Signal from the motor cortex to the muscles in red. Afferent signal from the receptors to the sensory cortex on grey. (Image extracted from webpage: http://andreeasanatomy.blogspot.com/2011/04/you-need-to-step-up-on-step-to-reach.html

The brain is a complex system of neurons, which are capable of sending information to different parts of our body, creating any movement or reaction, due to excitatory and inhibitory systems. Cellular receptors and neurotransmitters interactions can facilitate those system, regulating the level of neuronal excitability (Badawy, Loetscher, Macdonell, & Brodtmann 2012). Two of these neurotransmitter, which are important on the modulation of those systems, are Glutamate and γ-aminobutyric acid (GABA). Glutamate neurotransmitter is an excitatory neurotransmitter, meanwhile GABA

neurotransmitter is the major inhibitor in the human cortex (Badawy et al. 2012; Petroff 2002). Moreover, neurons, within the brain, have receptors that can modulate its own excitability, being *N*-methyl-D-aspartic acid (NMDA) one of those ones. NMDA can increase the excitability of the neurones through the interaction with glutamate. (Badawy et al. 2012; Blanke & Van Dongen 2009, pp. 283-329; Petroff 2002) Additionally, few studies have shown the importance of primary motor cortex receptor, on the potentiation of the synaptic activity and, therefore, increasing the effect of the long-term potentiation (LTP). (Bliss, Collingridge, & Morris 2004, pp 65-249; Bliss & Cooke 2011; Hasan et al. 2013)

Long- term potentiation (LTP) seems to be one of the two mechanism that leads to a short-term learning improvement. However, it does not seem to produce a long-term learning improvement, due to the balance between LTP and Long term depression (LTD) mechanism, which return, and balance, the initial values of the synaptic modification. Therefore, another mechanism must take over on the long-term learning, and this one is the synaptogenesis (Bliss et al. 2004, pp. 65-249; Bliss & Cooke 2011; Rosenkranz, Kacar, & Rothwell 2007; Rosenkranz, Williamon, & Rothwell 2007). This process consists on the adaptation of the neuronal system and brain to fulfil the demands of the motor task that has been trained for few days. Moreover, the reorganization of the brain regions involved in the movement will increase the synaptic strength. Thus, increasing the area of the brain that has been trained and the increase of synaptic connexions, enhancing synaptic responses. (Kleim et al. 2002, 2004; Rosenkranz et al. 2007)

As Bliss & Cooke (2011) suggest, LTP and LTD are mechanisms that enhance or reduce, respectively, the synaptic transmission through the activation of different receptors, enzymes and other intracellular signalling. Therefore, NMDA receptors and its location, in the motor cortex, are a combination which could modulate the behaviour in humans (Blanke & Van Dongen 2009, pp. 283-329). Thus, an increase in the synaptic transmission could produce LTP (Muellbacher et al. 2001; Rosenkranz et al. 2007; Rosenkranz et al 2007).

Yet, a great picture on how the corticospinal excitability regulates and, what is the centre governor that this excitability can come from has been described. Now, the understanding of how to modulate, artificially, that excitability and assess those systems and the changes that may or may not be created during different interventions. This will help to understand

how the nervous system response to this stimulus and support the use of the right protocol.

Nowadays, corticospinal excitability can be modulated with different equipment and different methods: Transcranial Magnetic Stimulation (TMS), Transcranial electric stimulation (TES)(Paulus, Peterchev, & Ridding 2013) and Transcranial Direct Current Stimulation (tDCS) (Madhavan, Sriraman, & Freels 2016).

Additionally, the most common and recent device to assess corticospinal excitability is, the beforementioned, TMS. This device will be described in, as well as how it works, its different methods and how it has been used to measure changes in corticospinal excitability in the latest researches focus on motor learning.

tDCS can facilitate LTP, through the increase of intracellular calcium, which is an effect of NMDA receptors. They are glutamate-gated cation channels with high permeability for Ca^{2+}, therefore, it will increase the facilitation of the corticospinal excitability (Blanke & Van Dongen 2009, pp. 283-329; Stagg & Nitsche 2011).

Moreover, it seems that tDCS can enhance brain activity and corticospinal excitability as a long term effect, consolidating the motor task (Ammann et al. 2016; Falcone et al. 2018; Jeffery et al. 2007; Kidgell et al. 2013; Stagg et al. 2018). It is done by reducing the activity of GABA receptors and increasing the activity of NMDA receptors, through the increase of Ca^{2+} influx into the postsynaptic neuron and, thus, inducing LTP (Ammann et al. 2016; Kidgell et al. 2013; Stagg et al. 2018).

Therefore, tDCS can produce an hyperpolarization or depolarization of the neurons, modifying the membrane action potential, and increasing or decreasing the likeliness of a neuron to fire an action potential (Sriraman et al. 2014; Stagg, Antal, & Nitsche 2018; Stagg & Nitsche 2011).

For a better understanding of the topic, this thesis will have a deep and extensive chapter about neurophysiology and how tDCS could increase motor learning and modulate cortical excitability.

Furthermore, the effect of the tDCS is affected by polarity positioning. Anodal transcranial direct current stimulation over the motor area during practise seems to increase motor learning and consolidation in upper limbs (Boggio et al. 2006; Buch et al. 2017; Reis & Fritsch 2011; Savic & Meier 2016; Stagg et al. 2011; Veldman et al. 2016) and lower limbs (Buch et al. 2017; Foerster et al. 2018; Schambra et al. 2011; Sriraman

et al. 2014).

Therefore, a dorsiflexion motor task could be learnt faster if tDCS would be apply on the motor area of the tibialis anterior, while performing the task. Nevertheless, intensity and current density are vital importance, as well as positioning. These parameters could increase certain physiological process of the learning that will be described in the following chapter. In this thesis, a different positioning of the cathode and a new motor task focus on the isolation of the muscle target will be used. Moreover, this thesis will oversee the effects of tDCS after a longer period of use and if there may be any residual effect after its use.

2. Literature review

2.1. Improvement of the accuracy and repetition of a movement over time

Learning and memory are a part of our daily life. The first one is the ability of changing a behaviour due to acquisition of knowledge about the world. The second one is the encoding, storage and retrieval of that knowledge (Kandel et al. 2013, pp. 1441-1459).

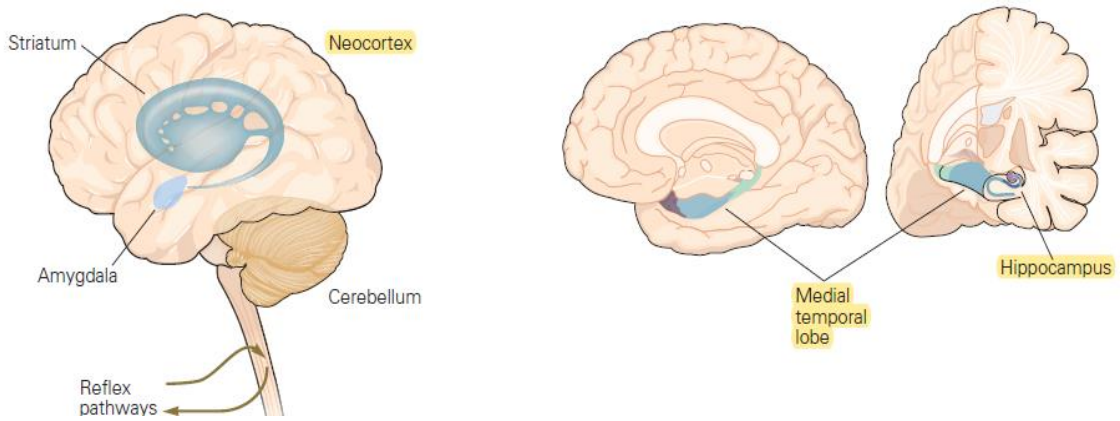

FIGURE 2. Representation of different parts of the brain involve in the memory process. extracted from (Kandel et al. 2013, pp.1462).

Few authors categorise motor learning as non-declarative, meaning that the movement is something that happen unconsciously(Chen et al. 2018; Huijgen & Samson 2015 and Song 2009). However, Song (2009), mentioned that motor learning should not be classify as non-declarative memory, as some movements requires a "conscious will" to create a sequence of movements that has an impact on the movement behaviour. Moreover, it seems that declarative or conscious memories are depending on a region from the brain, medio temporal lobe (MTL), that helps to create new memories traces that requires of consciousness (FIGURE 2) (Huijgen & Samson 2015; Song 2009).

Therefore, a good understanding of how memories are encode and storage, to be retrieved will help to oversee the procedures of learning that can be potentiated, through tDCS.

2.1.1. Memory as a part of learning

Memory is a process which involve 4 independent stages: generation/encoding, storage/stabilization, processing/consolidation and retrieval/maintenance. Each process

follows the other one. When a new stimulus take places, it generates a new trace formation. Then, this stimulus will need to get stable within the network of neurons, leading to the integration of the stimulus with other previous experience traces, and consolidate the pattern of neurons. Then, when this information is needed, the brain use the same trace and reinforce it. (Chen, Kam, Pettibone, Osorio, & Varga 2018; Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth 2013, pp. 1441-1485.; Rudy 2014, pp 151-396)

Few authors, (Chen et al. 2018; Huijgen & Samson 2015; Rudy 2014, pp 153-353 and Song 2009), talked about two different types of memory. One is declarative, that is related with explicit memory, in which the person is aware of the action. The other one is non-declarative memory, which is related to situations or abilities that happen when humans are not consciously aware of them, like motor learning. Declarative memory, can be subdivide into episodic memory, which can be related to a personal experience or events and semantic memory, which is related to general facts (FIGURE 3) (Huijgen & Samson 2015).

Two different areas of the brain are involved in the learning of the declarative memory, which are the medial temporal lobe (MTL) and neocortex (FIGURE 2) (Cartling 2001; Huijgen & Samson 2015; Lech & Suchan 2013). The medial temporal lobe, is composed by different structures including the hippocampus and parahippocampal gyrus, which is subdivide in perirhinal and the entorhinal cortex, which communicate the hippocampus with the neocortex (Huijgen & Samson, 2015; Lech & Suchan, 2013). This connexion is important to transform short term memory in long term memory (Kandel et al. 2013, pp. 1441-1520).

Nevertheless, different areas of the medial temporal lobe play a different role in the memory system, that is so, that once a memory has been acquired, this one can be repeated in a similar way without the involvement of consciousness. This is known as implicit memory, in which the outcomes are automatized, with the subject not being aware of the processing of the movement. Therefore, this memory does not differ from the condition originally learnt. Yet, there is another type, explicit memory, in which the subject is not only fully aware of the process but is, also, able to recall previous experiences and knowledge that have already learnt or practised. This kind of memory is more flexible,

since past experiences can be associated to resolve a new circumstance. (Kandel et al. 2013, pp. 1441-1520; Song 2009)

Episodic memory work on the explicit memory and has episodic and semantics forms (Kandel et al. 2013, 1441-1520). This memory works through the activation of certain patterns of synapses on the neocortical area and projecting it to the hippocampus, that is strengthened, forming a representation of a memory trace, on the hippocampus. Then, when a subgroup of synapses, like the initial projection is activated, will triggered the hippocampal representation. Thus, the hippocampus will stimulate those synapses that need to be fired to activate the entire pattern. (Rudy 2014, 285-396)

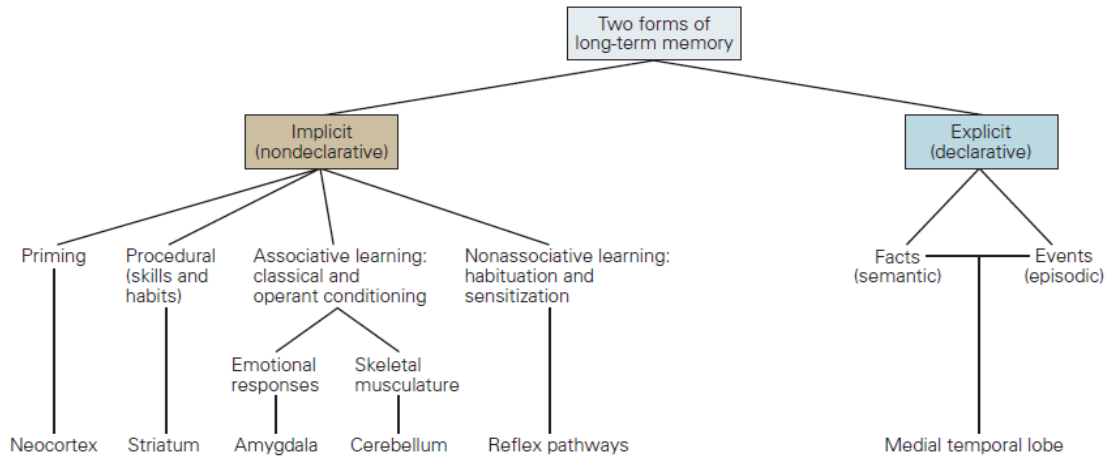

FIGURE 3. Classification of two forms of long-term memory extracted from (Kandel et al. 2013, 1446)

2.2. Neurophysiological basics to understand learning and memory

Getting to understand the neurophysiological process that is ongoing during implicit learning and memory will help to understand how the process could be modulated.

2.2.1. Neuron morphology

A neuron's morphology is tailor-made to receive, conduct and transmit signals. The dendrites have a great number of branches, with a large surface extension where they can receive the signal, this is the post synaptic area of the neuron. Then, the axon, which oversees the transmission of the action potential from the action hillock to the target cell, through the Nodes of Ranvier. Then, to propagate the action potential from one cell to another, the second one need to receive a current input that overreach its threshold. For

that, the presynaptic cell, is going to deliver, through neurotransmitter, an alteration in the membrane potential of the postsynaptic cell, which is the one that is going to receive them (FIGURE 4). (Enoka 2008, pp. 173-204; Kandel et al. 2013, pp. 21-333; Rudy 2014, pp 17-151).

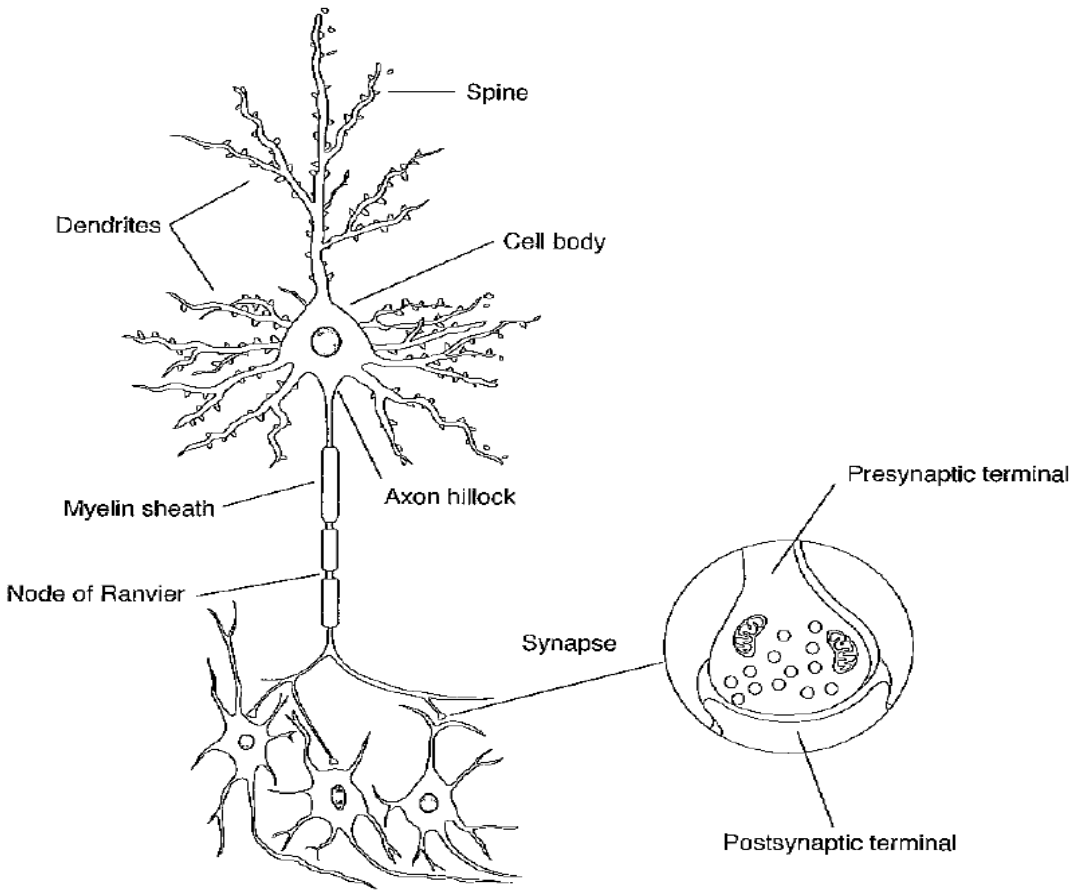

FIGURE 4. Neuron's physiology extracted from (Enoka 2008, pp 183)

There are two types of synapses: electrical and chemical. The second ones will use chemical transmitters to diffuse the action potential (Enoka 2008, pp 173-204; Kandel et al. 2013, pp. 21-333; Rudy 2014, pp 17-151).

Chemical synapses use a unidirectional transmission. The gap between pre-post synapses is bigger and the structure is composed by vesicles and active zones on the presynaptic and receptors on the postsynaptic. Thus, the transmission will be mediated by chemical transmitters. These synapses are more intricate and they have a greater variability of signalling than the electrical ones, producing more complex behaviours. These synapses can induce electrical changes in the postsynaptic cell, either inhibitory or excitatory

action, that can last from milliseconds to minutes. These are the synapses that predominate in the brain, due to small presynaptic neurons can modify the response on the postsynaptic cell, no matter its size. (Enoka 2008 pp. 173-204; Kandel et al., 2013 pp. 21-333; Rudy 2014, pp. 17-151)

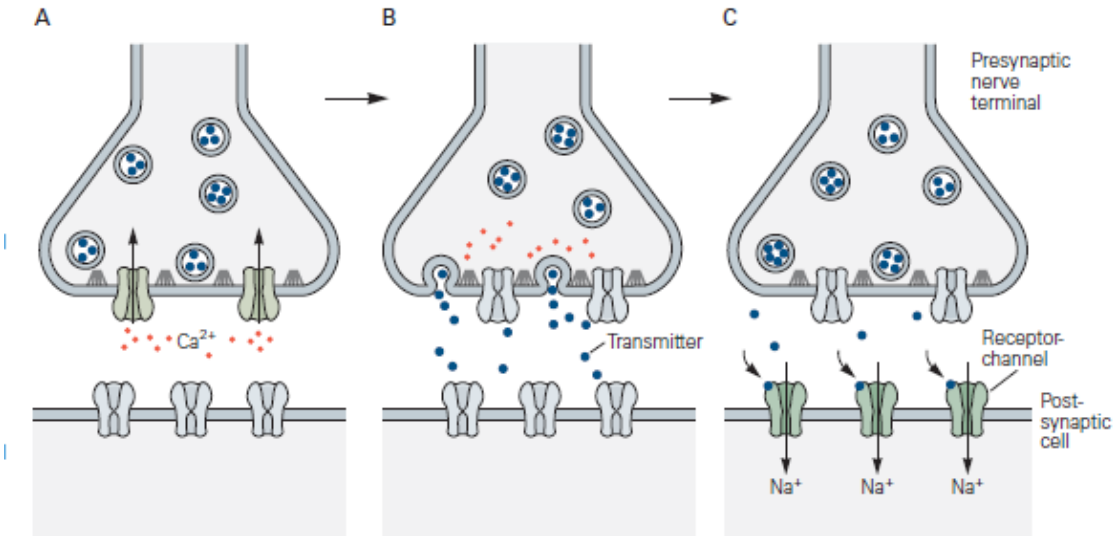

FIGURE 5. Synaptic transmission at chemical synapses extracted from (Kandel et al. 2013, pp. 185)

Furthermore, chemical synapses could be divided into two steps: the transmitting step, where the presynaptic neuron release different neurotransmitters, depending on the influx of Ca^{2+}, which are going to depend on the postsynaptic receptors. Then the second step, the receptive step, that is when the transmitter binds and activate the receptor molecules in the postsynaptic cell. Moreover, 2 types of receptors in the postsynaptic cell can be differentiated: ionotropic, also known as receptor-channel or ligand-gated channel and metabotropic receptors (FIGURE 5) (Enoka 2008 pp. 173-204; Kandel et al. 2013, pp 21-333; Rudy 2014, pp. 19-285).

2.2.2. Neuroreceptors and metaboreceptors

When a neurotransmitter bind into the ligand-gated channel, the ionotropic receptor experience a change on its structure. When the acetylcholine (ACh) is release from the synaptic boutons at the presynaptic neuron it travels through the synaptic cleft and binds the ACh gate receptors allowing Na^+ influx and efflux of K^+. The influx of Na^+ creates and imbalance at the resting membrane potential creating and action potential. They produce fast action lasting milliseconds. There are many ionotropic receptors, however,

NMDA, AMPA and $GABA_A$ are the most important to know, for its connection with corticospinal modulation. (Kandel et al. 2013, pp 21-333; Rudy 2014, pp. 19-285)

Metabotropic receptors, on the other hand, open ion channels through and indirect biochemical signalling pathway. The action of these receptors can last from seconds to minutes and modifies the neurons' excitability and the strength of the synaptic connection, modulating behaviour and producing long-lasting changes in the nervous system. Even though, there are not as many metabotropic receptors as ionotropic receptors, G protein-coupled receptor is one of the main ones affecting long-term potentiation. (Kandel et al. 2013, pp. 21-333; Rudy 2014, pp 19-285)

When a neuron generates an action potential to another, this generates a small excitatory postsynaptic potential (EPSP) on the postsynaptic neuron (FIGURE 6). This will make the post synaptic neuron more likely to fire an action potential again, by depolarizing the membrane temporarily, although there should be many EPSPs' to reach the threshold of the action potential. In contrast, neurons could be under a small inhibitory postsynaptic potential (IPSP) which is the opposite effect of the EPSP. This effect is caused by the excitation of an interneuron, producing a hyperpolarization. The IPSP could neutralized any excitatory action, even with the integration of many EPSPs, stopping the membrane potential to reach the threshold. (Enoka 2008, pp 173-204; Kandel et al. 2013, pp. 21-333; Rudy 2014, pp. 19-285)

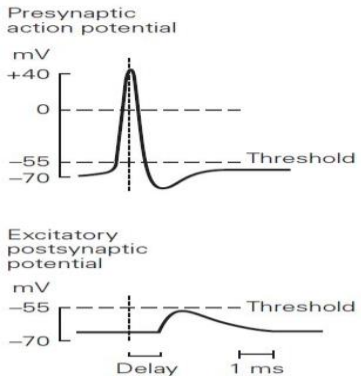

FIGURE 6. Presynaptic action potential and excitatory postsynaptic potential of a synaptic neuron extracted from (Kandel et al. 2013, pp. 185)

One of the most excitatory transmitter in the spinal cord and brain is the L-glutamate, being able to open glutamate-gate channels, which could create an effect on Na^+ and K^+ like the before mention acetylcholine (ACh), generating EPSP in the spinal motor cells. Thus, Glutamate is the main receptor for these neurotransmitters and, as mentioned before, there are ionotropic, which can be divide in AMPA, NMDA and kainate; this

thesis will focus on the 2 first. Then, the metabotropic receptors, G protein couple receptors, that will open channels indirectly through second messenger (FIGURE 7). (Enoka 2008, pp. 173-204; Kandel et al. 2013, pp. 21-333; Rudy 2014, pp. 19-285)

AMPA and NMDA receptors are situated in the postsynaptic membrane of most of the central synapses that use glutamate neurotransmitters. AMPA receptors are the predominant factor for the excitatory postsynaptic current, since it can generate a very rapid increasing and decreasing phase. While the NMDA receptor is the opposite, with a slow increasing and decreasing phase. This is because NMDA receptors has a Mg^{2+} blockage that is expelled when the membrane is depolarized. These receptors are also different than AMPA, because they also allow the extracellular Ca^{2+} to enter the postsynaptic neuron. This will produce a cascade of events that will be important in the long-term potentiation, reconstruction of the network of proteins at the postsynaptic density and long-term memory. (Kandel et al. 2013, pp. 21-333; Rudy 2014, pp. 19-285)

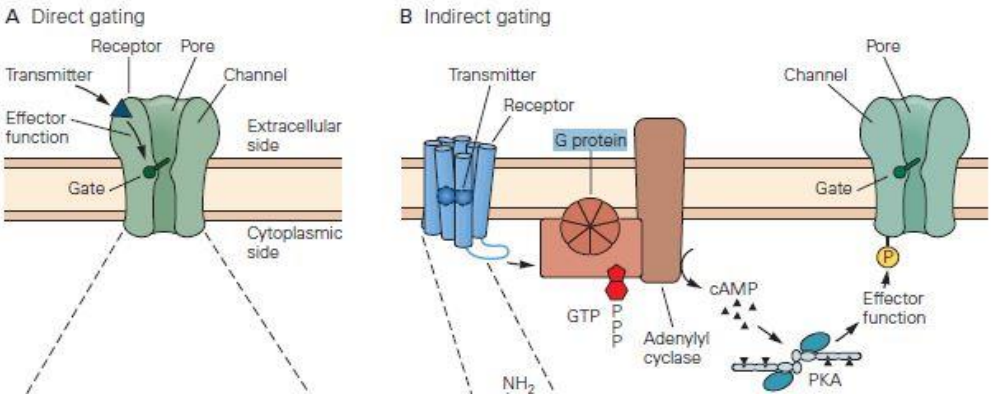

FIGURE 7. Direct and indirect gating on the postsynaptic membrane extracted from (Kandel et al. 2013, pp.187)

On the other hand, GABA neurotransmitter, which are the biggest inhibitor in the spinal cord and brain, producing inhibitory post syntactical potentials (IPSP). These neurotransmitters bind with the ionotropic receptor $GABA_A$ and the metabotropic $GABA_B$. The first one will open the Cl^- channels directly, while the second will use a second- messenger, that open K^+ channels indirectly. Opening Cl^- channels will increase the influx of Cl^-, producing a decrease on the membrane resting potential (from -65mV to -70mV), generating an increase on the total resting conductance of the membrane, therefore the EPSP depolarization will decrease, according to Ohm's law:

$$\Delta V_{EPSP} = I_{EPSP}/g_l$$

Where ΔV_{EPSP} is the amplitude of depolarization during EPSP, I_{EPSP} is the excitatory synaptic current and g_l is the total conductance of all other channels, including the Cl^-. (Kandel et al. 2013, pp. 21-333; Rudy 2014, pp.19-285) Also, $GABA_B$ response is slower and longer lasting than the $GABA_A$ (Kandel et al. 2013, pp 21-333).

Finally, the G protein couple receptors that can activate 2 different types of second messengers. Intracellular, which activity is related to the cell that they have been produced in; and transcellular, they can travel through the cells membrane to a neighbouring cell, acting as a first messenger or as intracellular signal. Yet, there are not many classes of G protein, different receptors can activate one type of G protein. The G protein can induce changes in a target protein, either phosphorylating it, through the action of a protein kinase or binding to it, through a second messenger. (Kandel et al. 2013, pp 21-333)

2.2.3. Mechanism of long-term potentiation.

When an action potential reaches the end of a presynaptic neuron it causes a release of a Glutamate neurotransmitter with the release of enough Ca^{2+} to create an EPSP. The Ca^{2+} is store in vesicles in the presynaptic neuron, and the action potential cause its release. When the concentration of Ca^{2+} is big enough, it will induce the release of the neurotransmitters. Then, the neurotransmitter will diffuse and bind to an AMPA receptor and NMDA receptors. Since, NMDA receptors has a Mg^{2+} blockage, they won't open until AMPA receptor creates an influx of Na^+, depolarising the postsynaptical membrane and expelling the Mg^{2+} blockage from the NMDA receptors. These NMDA receptors can bring extracellular Ca^{2+} into the postsynaptic neuron, which is going to trigger different second messengers that will produce changes in the membrane potential to the intracellular structures. (Kandel et al. 2013, pp. 21-333; Rudy 2014, pp. 19-285)

Once Ca^{2+} is coming inside the postsynaptic membrane it will trigger few events that will increase duration of LTP, depending on the number of theta-burst stimulation (TBS). If the TBS is low will give a form of short term potentiation ,triggering small release of intracellular Ca^{2+} and activating activate calpain proteins, this protein oversees the degradation and remodelling of the actin proteins, like spectrin, that crosslink in the postsynaptic density (PSD). (Briz & Baudry 2017; Rudy 2014, pp. 19-285) Moreover, ADF/cofilin works together with Calpain, targeting F-actin and G-actin, to disassembly them and then create a bigger structure, increasing the number of AMPA receptors and

enlargement of the postsynaptic density and, thus, of the dendritic spine (FIGURE 8). (Briz & Baudry 2017; Rudy 2014, pp. 19-285; Rust 2015)

In the other hand, if the number of TBS is higher, the release of Ca^{2+} will increase, and, thus, its concentration sending a second messenger of calmodulin, that with the help of Adenylyl cyclase, generates cAMP that activates PKA and MAP Kinase. Then, this will translocate to the nucleus, where it will phosphorylate CREB and initiate the transcription. If there is a repeated stimulation, it can activate translation in the dendrite

of mRNA through PKMζ, this will create more synaptic connections (FIGURE 8). (Kandel et al. 2013, pp. 21-333; Rudy 2014, pp 19-285)

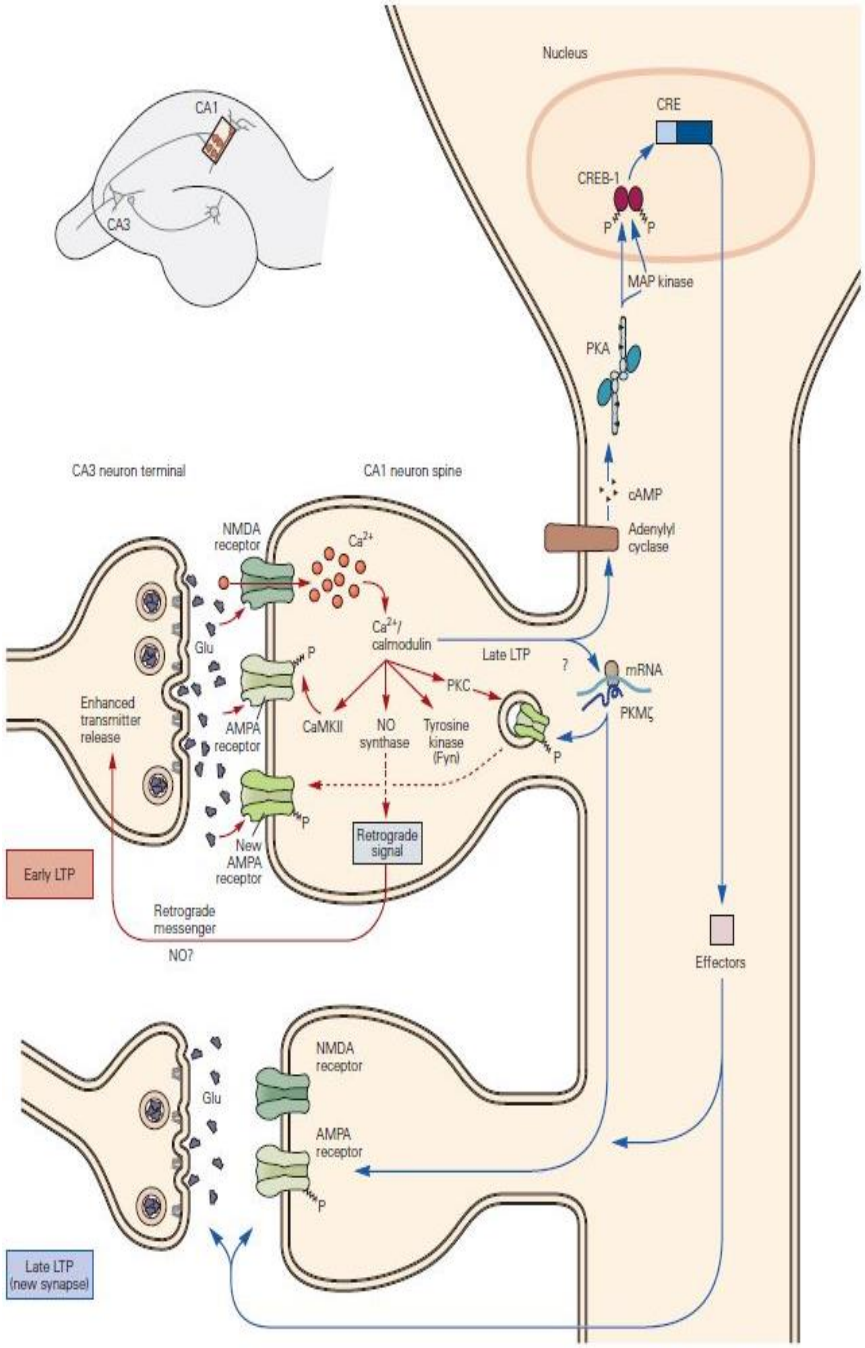

FIGURE 8. molecular mechanisms of early and late phase of long term potentiation model extracted from (Kandel et al. 2013, pp. 1553).

tDCS could increase the motor learning through the depolarization of the membrane, increasing Ca^{2+} into the postsynaptic membrane and triggering different process that produce an increase on NMDA receptors and connectivity between neuron. Therefore, a subject would reduce the rate of error on the motor task and cortical excitability may increase. This thesis measured the rate of error through a motor task and corticospinal

excitability with Transcranial Magnetic Stimulation, that can assess the excitability of the corticospinal tract.

2.3. Neurophysiological mechanics of Motor skill acquisition

2.3.1. Brain connectivity during motor learning

Fuster (2015, pp 237-293), cited that the motor skill learning probably follows the same principles as the perceptual networks applied to the prefrontal cortex. Specifically, prefrontal cortex may be connected to a complex system that includes posterior medial and orbital prefrontal areas; as well as hypothalamus, the anterior thalamus and the amygdala. These structures are important to evaluate the emotional significance of environmental events and for decision-making as well as mediate the formation of executive cognits in prefrontal cortex.

Kandel et al. (2013, pp. 1441-1520), brings up that prefrontal cortex has a high order connectivity with the motor cortex, which may enforce more variability on a context-dependent control over voluntary behaviour. Also, that many cortical motor areas are involve in the choice of the action that it should be taken. Specifically, primary motor cortex that is the area that generate simple movements, controlling the motor apparatus in the spinal cord. Then, the premotor cortex area will influence indirectly these movements with more complex and specialized commands. (Kandel et al. 2013, pp. 1441-1520)

Nonetheless, to create an input signal from the brain to the muscle to produce an action itself there must be a signal where our brain knows where is the spatial perception, attention and sensimotor information of head body and limbs. These efferent signals are process by the parietal lobe that will project the information to the prefrontal cortex, premotor cortex specifically, this will retrieve information from the hippocampus, creating a response through the motor cortex and send in it to the muscle through the corticospinal track. (Fuster 2015, pp. 237-293; Kandel et al. 2013, pp. 1441-1520).

2.3.2. Sensory feedback during motor learning

One of the most important sensimotor feedbacks to accurate control the movement is the visual feedback. Besides, the visual feedback provides information from two different streams: Ventral visual stream, which is the primary input and is limited to central vision;

and dorsal visual stream, which input is the full field of our eye sight, almost 180º. In the first one, ventral stream, the information requires focus, lighting and contrast, because this system is specialized in object identification and conscious perception of the environment, been related with vision for perception. In contrast, the dorsal stream is the opposite, it does not need much light or focus, this has been related with action. Thus, the ventral stream is on charge of recognition and identification, picking up information from

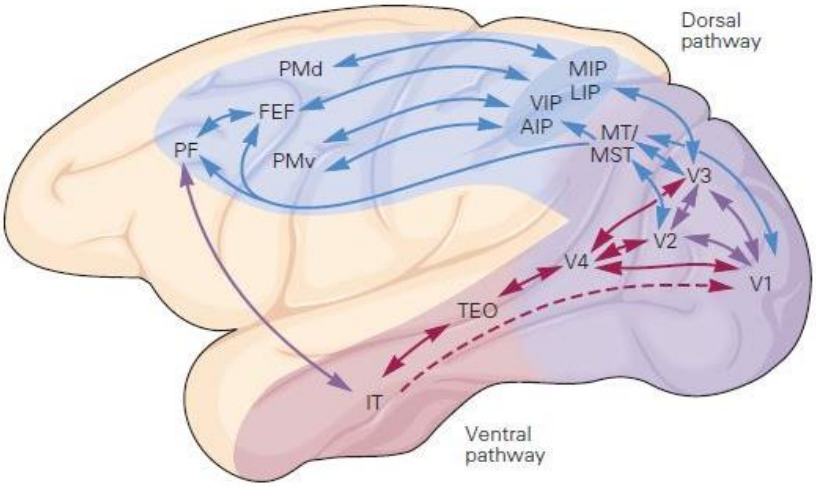

FIGURE 9. dorsal and ventral pathways involve in visual processing. AIP, anterior intraparietal cortex; FEF, frontal eye fields; IT, inferior temporal cortex; LIP, lateral intraparietal cortex; MIP, medial intraparietal cortex; MST, medial superior temporal cortex; MT, middle temporal cortex; PF, prefrontal cortex; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; TEO, occipitotemporal cortex; VIP, ventral intraparietal cortex; V1, V2, V3, V4, primary, secondary, third, and fourth visual areas. Extracted from (Kandel et al. 2013, pp 604).

the environment and storage it in the memory. Dorsal stream, on the other hand, integrate the information on how to control our motor system while interacting with and object. In FIGURE 9 different areas that these streams connect, and how they could interconnect with motor processing can be seen. (Schmidt, Lee, Winstein, Wulf, & Zelaznik, 2018)

In review, by mentioned that visual feedback reduces the time and increase the accuracy of an action through feedforwarding information about the unexpected situation, perceiving aspects of the environment, and the limb. Furthermore, vision feedback is a tool used to correct the direction of the movement that came through an unexpected disturbance and create a corrective submovement, thanks to control strategies, to perform error corrections in the available time (Khan et al., 2006). Also, it seems that is better having feedback after the task has been performed, as a form of knowledge or results (KR), letting the subject programme the movement for subsequent movements (offline Feedback) than during the performance of the task (online Feedback).(Khan et al., 2006; Schmidt et al., 2018) Moreover, instant offline feedback, given after practice, seems to

improve the mechanism of memory consolidation, compared to online feedback (Schmidt et al. 2018).

3. Transcranial direct current stimulation.

This technique is relatively new, since Nitsche & Paulus (2000) started to use it on humans, as a non-invasive and non-painful technique. However, this is not a new method, as Priori (2003) says, this technique has been applied since a long time, although it was painful and could cause brain bleeding, because the lack of control on the procedure and high intensity. Also, when electricity was not even discovered, doctors used to use an electric fish to relive people from different disease and pain. (Priori 2003)

Ttranscranial direct current stimulation (tDCS) is a non-invasive technique, which is based on a device made of a battery and a pair of electrodes, anode and cathode (FIGURE 10). The intensity of the stimulus should be between 1-2mA. (Cuypers et al. 2013; Kidgell, Goodwill, Frazer, & Daly 2013; Madhavan et al. 2016; Nitsche & Paulus 2000; Reis & Fritsch 2011) Moreover, depend on the position of the of the electrode, it is possible to increase or reduce the corticospinal excitability (Nitsche & Paulus 2000; Stagg et al. 2009).

FIGURE 10. tDCS device. Red Pad is the anode; Blue pad is the cathode and they are connected to the battery. Extracted from www.neurocaregroup.com

3.1. Positions of the transcranial direct current stimulation electrodes.

As can be seen in FIGURE 11, the position of the electrodes are really important, not just the placement of the anode and cathode, but the actual position in the M1, as well as the different positions that can be used. Nitsche & Paulus (2000) started looking for the best

placement on the motor cortex, but recent studies have shown that the best point is to focalise the area of the target muscle, using single pulse Transcranial Magnetic Stimulation (Kidgell et al. 2013; Madhavan et al. 2016).

Furthermore, the position of the anode and cathode plays an important role in this as well, being unilateral and bilateral positioning the most used for this device (Kidgell et al. 2013; Sehm, Kipping, Schäfer, Villringer, & Ragert 2013). These are the different positions:

- Unilateral: the active electrode is going on the Motor cortex area, precisely on the "hot sport" of the targeted muscle, and the reference electrode on a contralateral placement.
    - Anodal Stimulation: The anode is placed on the M1 area and the cathode will be placed over a contralateral placement, either the supraorbital (FIGURE 11 a)(Cuypers et al. 2013; Nitsche & Paulus 2000; Stagg et al. 2009), buccinator muscle(Avila et al., 2015) or shoulder (FIGURE 11 d)(Saucedo Marquez, Zhang, Swinnen, Meesen, & Wenderoth 2013; Schambra et al. 2011) . It increases the neuronal excitability, due to a neural depolarisation (Nitsche & Paulus 2000). This neural depolarization is the effect of a decrease in cortical GABA concentration (Stagg et al. 2009).
    - Cathodal stimulation: The cathode is situated in the M1 area and the anode in the contralateral supraorbital bridge (FIGURE 11 c) (Cuypers et al. 2013; Nitsche & Paulus 2000; Stagg et al. 2009) or shoulder (Schambra et al. 2011). It decreases the neuronal excitability, due to a decrease on the firing rate, produce for a reduction on glutamate release and, therefore, hyperpolarization of the postsynaptical potential (Stagg & Nitsche 2011).

- Bilateral: FIGURE 11 (b) shows the bilateral position. Where the anode electrode is placed on the hotspot of the target muscle, and the cathode on the contralateral hotspot of the motor cortex. (Kidgell et al. 2013; Mordillo-Mateos et al. 2012; Sehm et al. 2013) Mordillo-Mateos et al. (2012) found that this positioning produce an increase on the corticospinal excitability on the anode position, meanwhile it will reduce the interhemispheric functional connectivity of the contralateral motor area (Kidgell et al. 2013; Sehm et al.

2013). Moreover, Naros et al. (2016) has shown that the bilateral had a greater improvement of the motor performances than the unilateral.

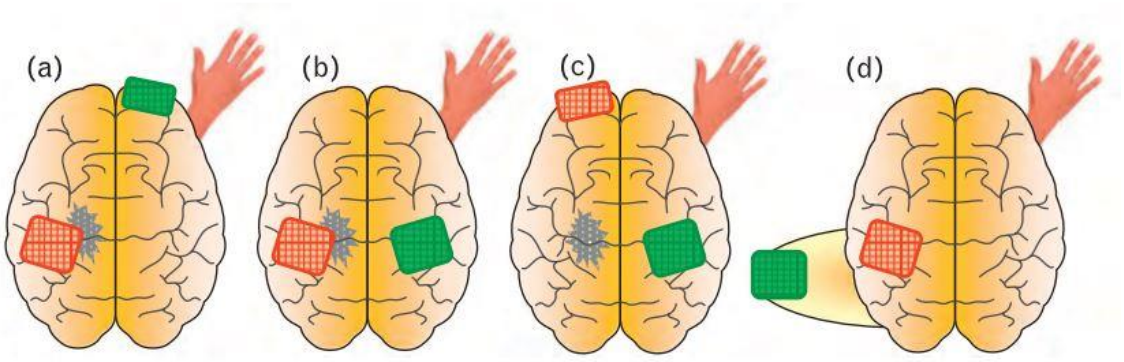

FIGURE 11. Positioning of the electrodes. Anode electrode (red and cathode electrode (green). Extracted from (Reis & Fritsch 2011)

3.2. Application timing and stimulation intensity of the transcranial direct current stimulation.

Although positioning is important, intensity and timing are factors that must be considered to ensure good quality on the application of tDCS. Few author suggested that the optimal intensity range is from 1mA to 2 mA (Cuypers et al. 2013; Jeffery, Norton, Roy, & Gorassini 2007; Kidgell et al. 2013; Madhavan et al. 2016; Nitsche & Paulus 2000; Reis & Fritsch 2011). Nitsche & Paulus (2000) defined 1 mA as the lowest intensity where difference on the corticospinal excitability difference can be seen. However, Cuypers et al. (2013) found different results using a 1.5 mA over 1mA, being 1.5 mA the intensity with the greatest improvement on performance, still, they also found changes on the 1 mA compared with the sham on the hand muscles. Furthermore, Jeffery et al. (2007) found that 2mA increase the corticospinal excitability even 60 minutes post-application.

These changes in intensity, could be because of the size of the electrode, reducing the side of the electrode, keeping the current density constant, can increase the focality of the tDCS. However, if the reference electrode increase, it will reduce the current density and it will make the tDCS inefficient and, also it will increase the depolarization of many areas of the brain (Nitsche & Doemkes 2007).

Moreover, timing or when the tDCS stimulation is given, either before or during the practise is also important. Few studies has demonstrated that tDCS enhance motor

learning, applied during the practise in either hand (Stagg et al. 2011) and in lower limbs (Sriraman, Oishi, & Madhavan 2014). On the other hand, if it is applied before the motor training it may cause and inhibitory process, which seems to be a decrease on the neuronal activity, due to a period of high synaptic activity, called homeostatic plasticity, slowing down the learning process (Sriraman et al. 2014; Stagg et al. 2011).

3.3. Electrode size and current density of the transcranial direct current stimulation.

Something related with the intensity is the electrode size and the current density of the subsequent modifications, this changes may have an effect in muscle specificity, discomfort and effectiveness of the device (Foerster, Rezaee, Paulus, Nitsche, & Dutta 2018; Nitsche & Doemkes 2007; Nitsche et al. 2003; Turi et al. 2014).

Nitsche et al. (2003) mentioned few safety consideration, in which includes few notes about current density, which is the result of the stimulation dived by the electrode size. This current density, should be below 25 mA/cm^2 otherwise will cause brain damage. However, in the literature, the highest value that has been used is 0.13 mA/cm^2(Avila et al. 2015; Shah, Nguyen, & Madhavan 2013; Sriraman et al. 2014) Furthermore, keeping this factor constant will increase the efficacy of the stimulation (Foerster et al. 2018; Nitsche & Doemkes 2007; Turi et al. 2014).

Also, it seems that keeping the current density constant and reducing the electrode size, not only reduce the cutaneous discomfort at same current intensity, but also increase the functional efficacy of the tDCS by increasing the spatial focality of the electrode (Foerster et al. 2018; Nitsche & Doemkes 2007; Turi et al. 2014)

3.4. Transcranial direct current stimulation and its effect in motor learning.

TDCS is a tool that has been used for a long time, in most of the cases to try to improve different mental diseases (Priori 2003). Nowadays the effect of this device has been studied either in cerebral stroke and Parkinson, which the motor cortex area is involved and, also in depression (Benninger & Lomarev 2010; Knechtel Lilly Thienel 2013; Mordillo-Mateos et al. 2012; Schlaug, Renga, & Nair 2009). Also, is being used to improve memory retention, isometric force and attention (Andrews, Hoy, Enticott,

Daskalakis, & Fitzgerald, 2011; Nelson, McKinley, Golob, Warm, & Parasuraman 2014). However, this review is considering the effects that this device has over motor learning and motor performance (Ammann, Spampinato, & Márquez-Ruiz 2016; Hashemirad, Zoghi, Fitzgerald, & Jaberzadeh 2016). Although, most of the papers has been research on, are based on simple movements, on the upper and lower body limbs, Zhu et al. (2015), used a golf putting task. Even though this research seems to improve the performance on the task, the research did not focus on the motor cortex areas, but in an area which affect to verbal analytic control.

Table 1-4 represent a guide of papers focus on the use of tDCS on either upper and lower body with a wide range of factors that can affect to either the performance and the modulation of the corticospinal excitability. This is probably because this device is quite new and not well researched on healthy subjects and either on the lower limbs.

Most of the papers in Table 1-4 used an intensity between 0.5 mA and 2mA, however, none of them use the optimal intensity that Cuypers et al. (2013) proposed of 1.5 mA. Moreover, despite the high intensity on few papers, the current density may be lower due to the electrode size (Devanathan & Madhavan 2016; Tanaka, Hanakawa, Honda, & Watanabe 2009) or even the tDCS electrodes placements (Vines, Cerruti, & Schlaug 2008). These changes, could make the difference when try to modulate the corticospinal excitability of the lower or the upper body. (Foerster et al. 2018; Kim et al. 2012; Nitsche & Doemkes 2007; Shah et al. 2013; Tanaka et al. 2009)

Furthermore, the placement is also important, not just to find the right hotspot, but also to places the references on the right places (Boggio et al. 2006; Kidgell et al. 2013; Saucedo Marquez et al. 2013). This is so important, that Saucedo Marquez et al. (2013) instead of placing the reference on the contralateral supraorbital area, she placed it on the extracephalical ipsilateral area, getting worse results than she expected, due to it might be less beneficial on motor skill learning.

Carring on with the positioning, another surprising fact is that even though, bilateral stimulation seems to increase cortical excitability and improve motor performance greater than unilateral (Foerster et al. 2018; Mordillo-Mateos et al. 2012; Sehm et al. 2013; Shah et al., 2013; Vines et al., 2008), most of the research on table 1-4 had used unilateral stimulation.

Furthermore, anodal tDCS can produce long term potentiation after 24h of application (Shah et al., 2013; Sriraman et al., 2014) and an increse on motor perfromance and force either after the practise session and after 3 days of motor practise (Saucedo Marquez et al., 2013). However, none of the above have developed a study that could produce synaptogenesis, with an intervention longer than 5 days.

Additionally, the limb involved and the side involved is also important, because different studies have shown that targeting the dominant hand has not shown greatest differences as when the non-dominant hand has been targeted. This consequence is due to an effect of the dominant hemipshere over the non-dominant, producing a celing effect on the dominant hand (Boggio et al., 2006). However, Boggio et al., (2006) kept the unilateral set up during both experiments, which produced an increase of corticospinal excitability on the non-dominance hand (Sehm et al., 2013; Vines et al., 2008).

TABLE 1. Motor learning and transcranial direct current stimulation in the upper body

Author	Year	tDCS set up	Limb	Methods	Task	Findings
Vines, B.W.; Cerruti, C. and Schlaug, G.	2008	Bilateral: anode right M1 cathode left M1 Unilateral: anode right M1 cathode left supraorbital	Left hand (non-dominant)	three different stimulation conditions on separate days (24h): bilateral, unilateral and sham 1mA during 20 minutes. C.D.: Bilateral .07 Unilateral .03	uni-manual pattern of five sequential keystroke as accurately as possible for 30 seconds	Bilateral stimulation increase the finger-sequence performance
Stagg, C.J.; Jayaram, G.; Pastor, D.; Kincses, Z.T.; Matthews, P.M; and Johansesn-Berg, H.	2011	Anodal stimulation and cathodal stimulation: left hemisphere M1 and contralateral supraorbital ridge	Right hand (not dominance has been mentioned)	Three different experiments with 3 different conditions on separate days: anodal stimulation, cathodal stimulation and sham. 1mA for 10 min Experiment 1 and 3: stimulation was before the practise Experiment 2: stimulation was during the practise and ongoing for 5 more minutes after the task ended.	Experiment 1: reaction time task. Marks were randomly shown in the screen on random interval time between mark. A total of 30 marks where shown. Experiment 2 and 3: explicit motor learning. They had to learn finger tapping sequence	Anodal stimulation during practise improve explicit motor learning and decrease reaction time
Boggio, P.S.; Castro, L.O.; Savagim, E. A.; Braite, R.; Cruz, V.C.; Rocha, R. R.; Rigonatti, S.P, Silva; M.T.A. and Fregni, F.	2006	Unilateral: anodal right hemisphere. Anode M1 right hemisphere. Cathode: contralateral supraorbital area	Experiment 1: Left hand (non-dominant) and experiment 2: right hand (dominant)	Anodal and sham stimulation on both experiments. 1mA for 20 min	Jebsen Taylo Hand function test before and after tDCS	Perfromance improvement on the non-dominant hand and not significant differences on the dominant hand

TABLE 2. motor learning and transcranial direct current stimulation on the upper body.

Author	year	tDCS set up	Limb	methods	task	findings
Saucedo-Marquez, C.M.; Zhang, X.; Swinnen, S.P; Meesen, R. and Wenderoth, N.	2013	Unilateral: anodal stimulation anode right hemisphere cathode: extracephalical ipsilateral area	Left hand (non-dominant)	3 days training protocol+retention test Anodal and sham stimulated on both experiments 1mA during 20 min	Sequential finger tapping task and isometric force control task	Anodal group was greater on sequence tapping from day 1-3. force improved but not significant differences were found
Kidgell, D.J.; Goodwill, A.M.; Frazer, A.K. and Daly, R.M.	2013	Unilateral: anode right M1 hand muscle cathode contralateral supra orbital area. Bilateral stiulation: anode on the right M1 of the hand muscle and cathode on the left representation of the hand muscle	Left hand (non-dominant)	1 day unilateral, bilateral and sham stimulations 1mA for 13min	Picking up small pegs and place them on a vertical array of holes using index finger and thumb.	Bilateral stimulation reduce SICI further than unilateral, however same improvements has been shown in motor learning.

TABLE 3. motor learning and transcranial direct current stimulation on the lower limbs

Author	year	tDCS set up	Limb	methods	task	findings
Sriraman, A.; Oishi, T., and Madhavan, S.	2014	Unilateral: anode TA M1 area. Cathode over the contralateral supraorbital area	Left leg (non-dominant)	One day each condition separate by 7 days: anodal before training, anodal after and sham. 1mA for 15 min	Ankle dorsi and plantarflexion with a device, following a sinusoidal wave from display on a computer screen	tDCS during task improve motor performance. However, there were no significant changes on the corticospinal excitability in the 3 different conditions.
Devanathan D, Madhavan S	2016	Unilateral: anode TA M1 right hemisphere cathode contralateral supraorbital region	Left leg (non-dominant)	One day each condition separate by 7-9 days: anodal and sham during. 1mA for 15 min. Current density: 0.08mA/cm2	Motor tracking task with the ankle.	No changes on RT either on upper or lower limbs and either changes in cognitive function
Tanaka, S.; Hanakawa, T. Honda, M. Watanabe, K.	2009	Unilateral: anode TA right motor cortex hemisphere cathode contralateral orbit	Left leg (non-dominant)	One day each condition separated by 1 week: anodal, cathodal and sham. 2mA during 10 min with a current density of 0.057 mA/cm2	Pinch force task, and reaction time task either with hand and with leg	Increase in pinch force on the lower limb but not on the hand muscle. Reaction time was not change.

TABLE 4. Motor learning and transcranial direct current stimulation on the lower limbs.

Author	year	tDCS set up	Limb	methods	task	findings
Foerster, Águida Dutta, Anirban Kuo, Min Fang Paulus, Walter Nitsche, Michael A.	2018	Unilateral: anodal TA and cathodal to the above to the contralateral	Right leg (dominant)	Experiment 1: One day and retention test. Anodal and sham conditions. 0.5 mA during 15 mins with a current density of 0.056 mA/cm2 Experiment 2: same condition but 2 mA intensity. Keeping Current density	Isometric visuomotor task	Better performance for Stim after 24 hours. However, Individual characteristics, sensitivity to TMS and stimulation protocol
Shah, Bhakti Nguyen, Tai Tri Madhavan, Sangeetha	2013	Unilateral: anode: Cerebellum(left) and M1(right) cathode: ipsilateral left buccinator(cerebellum)and contralateral forehead(M1). Anodal, cathodal and sham.	Left leg (non-dominant)	tDCS conditions are 1 mA during 15 mins. Current density of 0.125	Visuomotor task	Cerebellum anodal, cathodal and M1 anodal had similar modulation effect.

4. Transcranial Magnetic Stimulation as a measurement of corticospinal excitability in motor learning.

Barker, Jalinous, & Freeston (1985) introduce Transcranial Magnetic Stimulation (TMS) as an instrument (Figure 12) to stimulate the motor cortex with a non-invasive technique and able to elicit Motor Evoke Potential (MEPs). It was a great advance on the technique, because it, also, minimized the discomfort of the stimulation, compared with the conventional once, brought up as a Transcranial Electrical Stimulation (TES) by Merton & Morton, (1980) few years before.

FIGURE 12. transcranial magnetic stimulator. Extracted from https://www.magstim.com

This technique was developed to associate the different parts of the body with different areas of the motor cortex (Cohen & Hallett 1988; Wassermann, McShane, Hallett, & Cohen 1992), through a magnetic field, generated on a wire coil (Rotenberg, Horvath, & Pascual-Leone 2014, pp. 3-57), which elicit a motor response of the contralateral peripheral motor neurons due to an action potential that goes down the corticospinal tract (Cavaleri, Schabrun, & Chipchase, 2015). This creates an electrical potential on the muscle cells, that it can be measure with an electromyograph (EMG), and is called motor evoke potential (MEPs) (Badawy et al. 2012; Cavaleri et al. 2015; Rotenberg et al. 2014, pp 3-57). Moreover, changes in MEPs can indicate different alterations on the corticospinal tract and neuronal network (Badawy et al. 2012; Cavaleri et al. 2015).

TMS has been developed since it has been created, introducing different paradigms, pulse waveforms, pulse strength and different magnetic coils to focalise the stimulus on the

motor cortex areas of interest and to use the right technique to study the phenomenon of interest (Badawy et al. 2012; Cohen et al. 1990; Rotenberg et al. 2014, pp. 5-57)

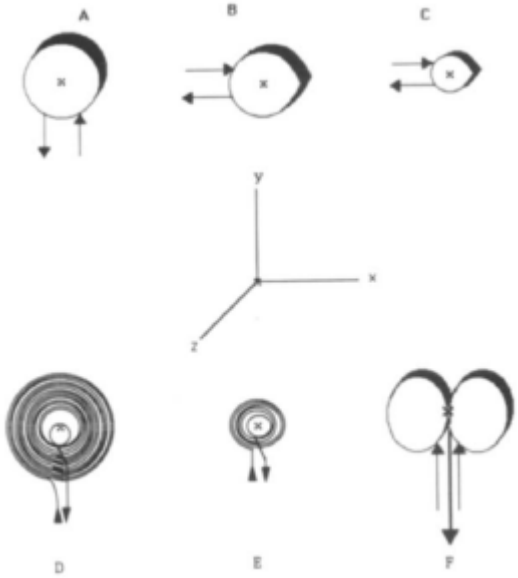

FIGURE 13. Magnetic Coil working mode. Extracted (Cohen et al. 1990)

In relation to the magnetic coil Cohen et al. (1990) evaluated the focalization of different magnetic coil shape and the focalization of them as well as the peripheral nerve stimulation. It creates a magnetic field due to the current that its spinning around the coil (FIGURE 13), this magnetic field generate a current in the opposite direction on a nearby conductor (Rotenberg et al. 2014, pp. 5-57). However, a wide variety of shapes and size of magnetic coils can be found, which is important for the focality of them (Cohen et al. 1990; Rotenberg et al. 2014, pp. 5-57).

4.1. Transcranial Magnetic Stimulator Coil to assess corticospinal excitability

Rotenberg et al. (2014, pp 5-57) made a classification of the different types of coils, illustrated in FIGURE 13 and FIGURE 14.

FIGURE 14. Coil shapes. (from right to left). Circle coil, figure 8 coil, double cone doil, H-coil. Extracted from Rotenberg et al. (2014, pp 5-57)

- Circular or Round Coil: Although Barker et al. (1985) used this coil for the first time, founding that it was more focal and less painful than the ones they were used at the time. Nowadays has been relegated to the least of the uses, because is not very focal. It is used for single pulses protocols and peripheral stimulation. (Cohen et al., 1990)
- Figure 8 Coil or butterfly: This Coil is more focal than the first one, because it has 2 circle coil creating electrical fields on opposite directions, therefore the focality of the coil is greater than the one before (Cohen et al. 1990). However, it seems that this coil cannot reach deep areas of the brain due to its shape and electric field centre (Zangen, Roth, Voller, & Hallett 2005).
- Double Cone Coil: The shape of this coil is like the figure-8 ones but it has a bent angle, as can be seen in FIGURE 14. This one can reach deeper areas than the Figure 8 coil (Lontis, Voigt, & Struijk 2006), but, they need greater intensities than the H-coil (Zangen et al. 2005).
- H-Coil: This one is the most complex one, due to its design. Despite the fact that it can reach deeper areas of the brain, making it greater than the H-coil to reach the lower limbs areas of the motor cortex, it is not that focal as the figure 8 Coil (Zangen et al. 2005).

4.2. Paradigms used with Transcranial Magnetic Stimulation in motor learning

Rotenberg et al. (2014, pp. 69-129) define that there are a lot of methods that you can use with TMS. The most interesting for this review is the Single pulse and Paired pulse paradigms.

- Single Pulse paradigm: Barker et al. (1985) already used this method and consist on a Single pulse of TMS applied over the motor cortex that will produce a stimulation of the target muscle and a electromyography EMG response on form of Motor Evoke potential (MEP). The intensity, plays a big role in this paradigm, been the one which trigger the MEPs, suprathreshold or not getting any at all, subthreshold. Information of the corticospinal excitability can be obtained due to the different intensities of the MEPs and thresholds. Some of the protocols are: motor threshold (MT), input/output curve, contralateral silent period (cSP), and ipsilateral silent period (iSP) (Di Pino et al. 2014; Rotenberg et al. 2014, pp. 69-129).

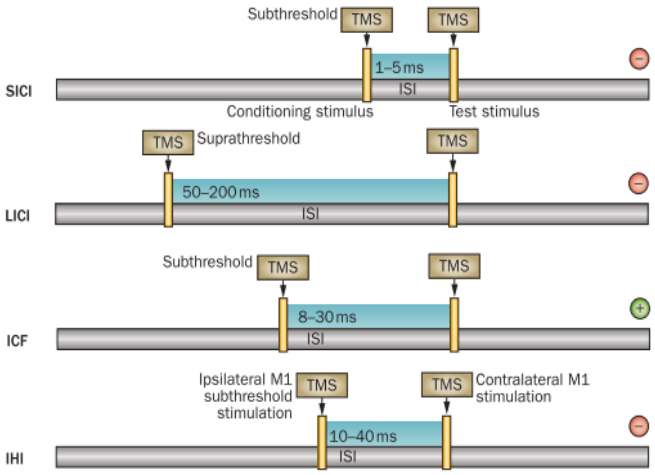

FIGURE 15. paired pulse protocols. Red circle: protocols to measure inhibition; Green circle: protocols to measure facilitation. Modify from Di Pino et al. (2014)

- Paired- pulse Paradigms: This technique consists on the delivery of two consecutive stimuli on the same point of the brain. In this technique, the effects of the first pulse, conditioning stimulus (CS), on the cortical pathway produce changes that can be measure by the variations of the second one, test stimulus (TS). Moreover, the intensity of each stimulus and the interval time between them (ISI) must be chosen carefully, because the imact that they can cause in different circuits. (Ferreri et al. 2011; Rotenberg et al. 2014, pp) The different protocols are: Short-interval intracortical inhibition (SICI), Long-interval intracortical inhibition(LICI), Intracortical facilitation (ICF) and Interhemispheric inhibition (IHI) (FIGURE 15) (Di Pino et al. 2014).

4.3. Different transcranial magnetic stimulation methods used to assess corticospinal excitability in motor learning.

Before going in depth with one protocol of each paradigm, a single pulse protocol that is important on the understanding of the paradigms mas be consider. This protocol tries to identify the motor threshold of the corticospinal pathway, due to a transcranial magnetic stimulation, which will produce a Motor Evoke Potential (MEP) response on the EMG (Rotenberg et al. 2014, pp. 3-129; Westin, Bassi, Lisanby, & Luber 2014).

Moreover, the motor threshold has two ways of measure, depending on the muscle tension of the muscle target.:

- Rest Motor Threshold (rMT): when the muscle targeted is at rest the intensity of the stimulus must be the lowest capable to see a MEP, although the peak-to peak amplitude has to be bigger than 50μV (Kaelin-Lang et al. 2002; Maeda, Gangitano, Thall, & Pascual-Leone 2002; Maeda, Keenan, Tormos, Topka, & Pascual-Leone 2000; Westin et al. 2014).
- Active Motor Threshold (aMT) when the muscle is at a % of the muscular voluntary contraction (Boroojerdi et al., 2000; Rotenberg et al., 2014). Also, the peak-to-peak amplitude must be greater than >200 μV (Rosenkranz et al. 2007; Rosenkranz et al. 2007; Temesi, Gruet, Rupp, Verges, & Millet 2014).

4.3.1. Input/output Curve with transcranial magnetic stimulation in motor learning.

The literature also defines it as “recruitment curve” (RC) or as “stimulus-response curve (SR) (Lotze, Braun, Birbaumer, Anders, & Cohen 2003; Rosenkranz et al. 2007; Rotenberg et al. 2014, pp. 69-117 ; Temesi et al. 2014). It is a single pulse paradigm, which measures the MEPs on a wide range of intensities of the Motor threshold (Lotze et al., 2003; Rosenkranz et al. 2007; Rotenberg et al. 2014, pp. 69-117) or % of the MVC (Temesi et al. 2014). The normalization of the MEPs gather from those intensities will increases exponentially until reach a plateau (Figure 16). The selection of the intensities

use to be subthreshold to observe better increases on the I/O curve (Avanzino et al. 2015; Boroojerdi et al. 2000; Rotenberg et al. 2014, pp. 69-117).

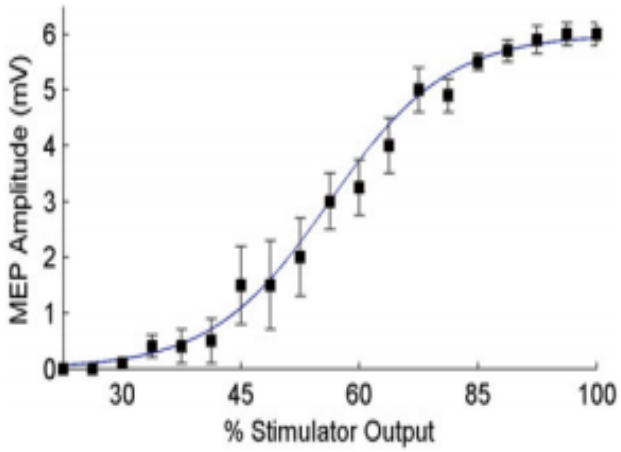

FIGURE 16. Input output curve(Rotenberg et al. 2014, pp 91)

4.3.2. Short-Interval Intracortical inhibition with transcranial magnetic stimulation on motor learning.

SICI is a pair pulse paradigm, which activates $GABA_A$ receptors and produce inhibition, decreasing the excitability of the cortical pathways. Furthermore, as mentioned before, this paradigm has two intensities, one is subthreshold (Conditioning stimulus) and the other one is the suprathreshold (Test stimulus) (Badawy et al. 2012; Rotenberg et al. 2014, pp. 117-129). The motor threshold depends on the aim of the study and could be either active (Kidgell et al., 2013; Rosenkranz et al., 2007) or at rest (Berghuis et al. 2016; Perez, Lungholt, Nyborg, & Nielsen 2004; Veldman, Zijdewind, Maffiuletti, & Hortobágyi 2016). The intensities between 70-90% are consider in the literature (Bastani & Jaberzadeh 2013). Besides, the interstimulus interval (ISI) is quite important to focalise the right mechanism (Rotenberg et al. 2014, pp. 117-129), and for this protocol tend to be between 1-4 ms, which produce a suppression on the respond (Kujirai et al. 1993; Ziemann, Rothwell, & Ridding 1996).

5. Purpose of the study

5.1. Introduction

Transcranial direct current stimulation (tDCS) is a device use to apply a non-invasive current on the skull, from 0.2 mA-2mA(Cuypers et al. 2013; Nitsche & Paulus 2000; Nitsche et al. 2003) and its use has been increasing in different areas, due to its apparent benefits (Buch et al. 2017; Kang, Summers, & Cauraugh 2016; Nitsche & Paulus 2000; Schlaug et al. 2009). Specially, when applied on the motor cortex there is an increase in performance (Vitor-Costa et al. 2015), learning (Falcone, Wada, Parasuraman, & Callan 2018) and motor learning (Buch et al. 2017).

Moreover, anodal transcranial direct current stimulation over the Motor area during practise seems to increase motor learning and consolidation in upper limbs (Boggio et al. 2006; Buch et al. 2017; Reis & Fritsch 2011; Savic & Meier 2016; Stagg et al. 2011; Veldman et al. 2016) and lower limbs (Buch et al. 2017; Foerster et al. 2018; Schambra et al. 2011; Sriraman et al. 2014).

Moreover, it seems that tDCS can enhance brain activity and corticospinal excitability as a long term effect, consolidating the motor task (Ammann et al. 2016; Falcone et al. 2018; Jeffery et al. 2007; Kidgell et al. 2013; Stagg et al. 2018). It could reduce the activity of GABA receptors and increasing the activity of NMDA receptors, through the increase of Ca^{2+} influx into the postsynaptic neuron and, thus, inducing LTP (Ammann et al. 2016; Kidgell et al. 2013; Stagg et al. 2018). However, most of the research about changes in corticospinal modulation and brain activity has been done in the upper body (Avanzino et al. 2015; Fricke et al. 2011; Kidgell et al. 2013; Nitsche & Paulus 2000) and few in the lower body (Jeffery et al. 2007; Shah et al. 2013).

However, there is still some gaps on how anodal tDCS affect to consolidate a motor task, reducing the rate of error, in the lower limbs and if it produces any modification on the corticospinal excitability. Tibialis anterior will be the muscle targeted, using an isometric dorsiflexion force to track a sinusoidal curve. Moreover, motor task and positioning have not been used in any study before. Thus, this thesis is focus on how tDCS would affect the rate of error and corticospinal excitability with a different positioning. Therefore, the

hypothesis, is that applying unilateral tDCS over the M1 and cathode over the contralateral shoulder during 5 days' period will increase corticospinal excitability, reduce intracortical inhibition and enhance motor learning and consolidation.

6. Methods

6.1. Participants

Fifteen healthy participants (9 males and 6 females age: 26.2±4.5; height: 177 cm; weight: 70 kg) took part in the experiment. Two subjects (1 female and 1 male) had to drop out of the study due to an increasing tinnitus symptom. One male subject could not participate on the retention part of the study. The exclusion criteria included the presence of metal implants in the head, neck and heart, any neurological and psychiatric disorders, as well as the use of medication and substance that could have an influence the nervous system. Also, participants had to be right foot dominant, and it was assessed by kicking a football.

Participants were assigned to a group depending on sham condition (6 subjects) and stimulus condition (7 subject, although one of them did not do the retention test). The groups were randomised and organised by people outside the research project, changing the condition as the participants came to the test or training. Neither the participants or the researcher knew the condition, being a double-blind study.

The subjects were voluntary participants that were fully informed of the experimental procedure. They could drop out the study at any time. They were provided with an informed consent approved by the University of Jyväskylä ethics committee and conformed to the Declaration of Helsinki.

6.2. Experimental design

Participants came to the lab for a total of 6 times, 5 days in a row and a retention day, which was 8 days after the last training day. On the first day, maximal isometric voluntary contraction (MIVC) and different TMS measurement that will be describe later were assessed. These measurements were realised pre-and post-intervention on day one, day five and day six (retention test). The intervention part was done between measurements and days 2-4. The training was done with 2 different TDCS conditions: SHAM condition (placebo) and STIM condition (stimulation group). The participants had to match a sinusoidal curve applying between the 20-50% of the MIVC. The training consisted in 3 sets of 4 minutes of practice with 1 minute of rest. During those 4 minutes, the subject

had a 10 seconds' offline motor practise and 20 seconds' rest, where they could get feedback from the performance.

The task consisted in a dorsiflexion isometric force task performed with the Tibialis Anterior (TA) muscles following a sinusoidal curve displayed on a computer screen FIGURE 17. The sinusoidal curve started at the 35% of the MIVC, up to the 50% and down to the 20%, a whole cycle was completed on a second. They started at the sign of "go" and they stopped at the signal of "stop". The signal was given always at the lowest point of the sinusoidal curve of the sinusoidal curve. During the resting part the subjects were given an offline feedback, in which the researcher facilitates on a screen the performance of the subject overplayed on the target.

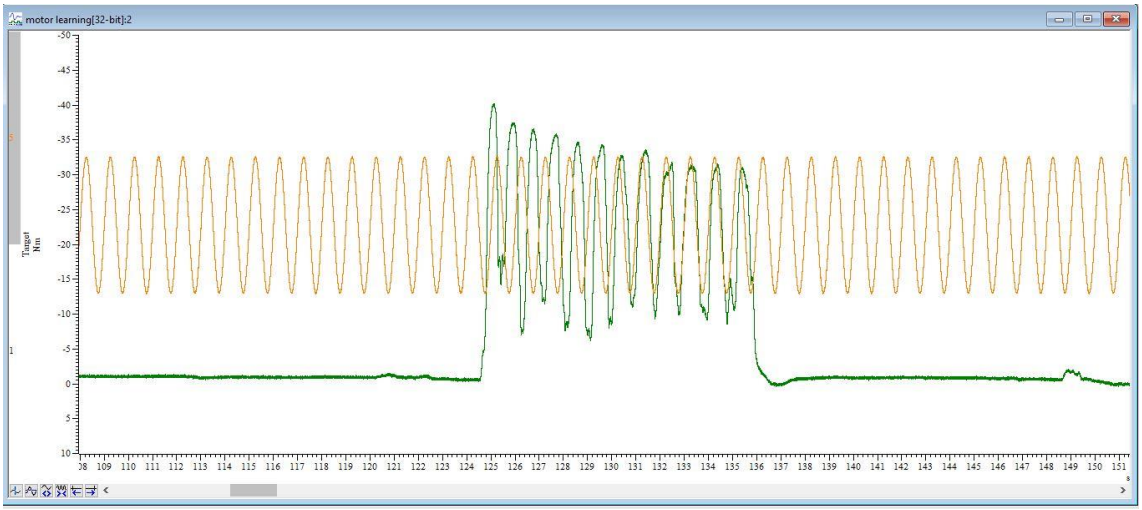

FIGURE 17. Motor learning task (orange line) and force track from the isometric force applied with the tibialis anterior dorsiflexion(green line). Extracted from Spike2 demo version

TDCS electrodes were covered by sponges soaked in a saline solution at the beginning of the training to avoid an increase of the resistance of the electrodes. Moreover, after every set, 5 ml were added in both sponges to keep the resistance <8 kΩ

MIVC was measured every day, adjusting the sinusoidal curve to the condition 20-50% MIVC. This adjustment consisted in assessment of the greatest MIVC out of 2 trials, separated by a 1 min rest. The MIVC were performed after a warm up of 2 sets of 2 trials of different intensities with a rest period of 30s between each other.

Subjects were seated with the knee joint fully extended, the hip joint at 120° of extension, and the ankle joint at an initial position of 90° (i.e., the sole of the foot at right angles to the tibial axis) in an ankle dynamometer (Neuromuscular Research Center, University of

Jyväskylä). All measurements were performed on the left leg while the right leg rested quietly on a support. The left foot was firmly attached to a footplate mounted on the rotation platform so that the rotation axes of the ankle joint and the motor-driven platform coincided. The length of the leg was also measured to ensure the participants had the same position during the whole experiment. An additional strap with a foam support prevented the right knee joint from flexing. The torque around the rotational axis of the motor was measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland) Torque signal was sampled at 1 kHz with a 16-bit A/D converter [CED power 1401, Cambridge Electronics Design (CED), Cambridge, UK] and stored for later analysis FIGURE 18.

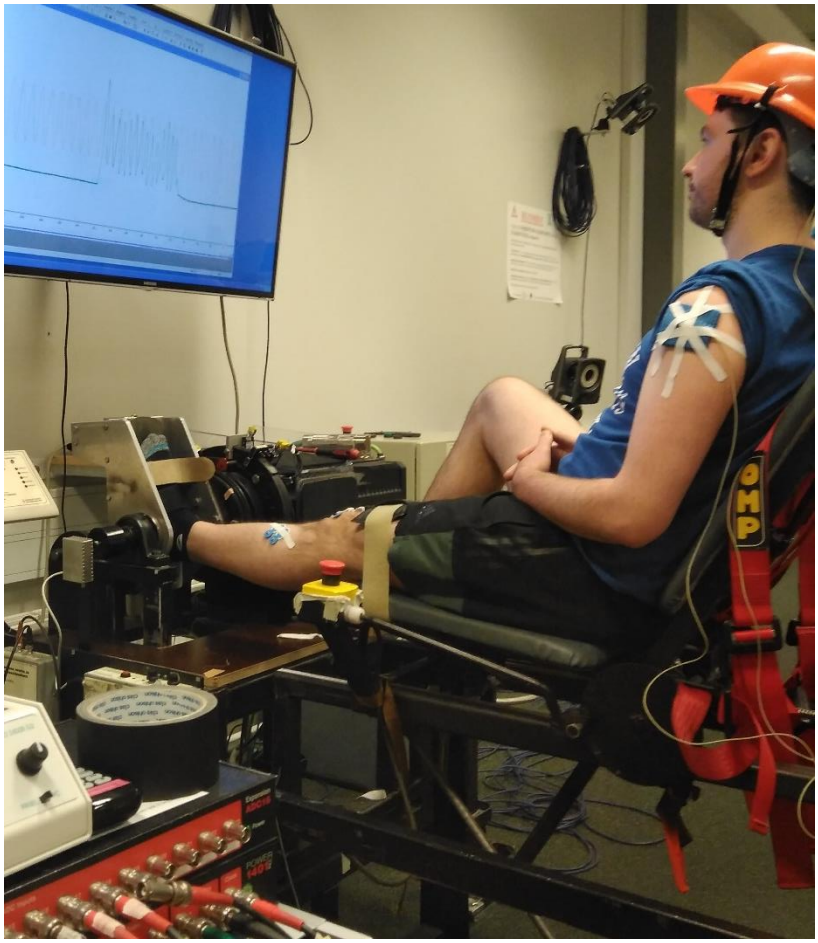

FIGURE 18. Positioning of the subject during the measurements and motor task.

All measurements were assessed on the same leg extension and ankle position before mentioned. Isometric maximal voluntary contraction was assessed before the corticospinal measurements. This was done with a previous warm up of 2 sets of 2

repetition of two different increasing intensities, separate of 30 second one from each other. Participants were asked to performed 2 isometric voluntary contractions for at least 3 seconds, with a 1 min break. While performing the MVC participants were encourage to produce the maximal force. Trials with a countermovement were excluded. If both attempts had a countermovement, subject could try a 3rd time. After, the attempt, the greatest one was chosen.

Then, assess the corticospinal excitability was assessed, with 2 different TMS protocols. Single and double pulse protocols. Subject was asked to come out of the chair for 2 minutes between protocols, since the position could create some numbness on the left foot or fatigue. Testing sessions did not take more than 120 mins, including the intervention part and setting the tDCS.

7. Measurements

7.1. Electromyography

EMG activity was recorded from tibialis anterior (TA) and gastrocnemius medialis muscles of the left leg with bipolar self-adhesive electrodes (Blue Sensor N,Ag/AgCl, 0.28 cm2; Ambu, Ballerup, Denmark), and a ground electrode was placed on the patella. Electrode placement and skin preparation were performed according to SENIAM (Hermens et al. 2000). Reference lines were drawn on the skin, and a picture was taken to provide accurate replacement of the electrodes in the following sessions. The electrodes were adjusted on the muscle belly in accordance with the underlying fibre direction (interelectrode distance=2 cm; interelectrode resistance<2 kΩ). Alignment of the electrodes was checked according to the EMG activity when the participant was asked to pull and push, which was ensured to be smooth during the warm up previous to the MIVC. EMG signals were amplified and high-pass filtered (1,000x, 10 Hz) by a preamplifier (NL824; Digitimer, Welwyn Garden City, UK) and then band-pass filtered (10 Hz to 1 kHz) by a differential amplifier (NL900D/NL820A; Digitimer). The signals were acquired on a personal computer at a rate of 5 kHz via a 16-bit A/D converter (CED power 1401; CED).

7.2. Transcranial magnetic Stimulation

TMS was delivered with a single-pulse, monophasic Magstim 2002 stimulator with a 9-cm double batwing coil (Magstim, Whitland, UK), oriented to deliver posterior-anterior directed current to the motor cortex. The coil was optimally positioned to elicit at rest Tibialis anterior (TA) MEPs with the greatest amplitudes while eliciting minimal gastrocnemious medialis (50% of TA MEP amplitude). Marks were drawn on the subject's scalp to facilitate monitoring of coil position throughout the testing session and to enable accurate coil repositioning in the following sessions.

Resting motor threshold (RMT) was defined as the lowest stimulus intensity to elicit a visible MEP with peak-to-peak amplitude of >50μV in three of five consecutive trials. (Avanzino et al. 2015; Paulus et al. 2013) RMT was calculated to set the intensities for the Input/output curve (I/O curve) which would be between 100-140% of the RMT, with an increase of 10% intensity each time. At least 5 out of 10 stimulus were >50μV

(Avanzino et al. 2015; Rosenkranz et al. 2007). Ten-twelve stimulus were given in each intensity of the RMT

Secondly, active motor threshold (AMT) was defined as the lowest stimulus intensity to elicit a visible MEP with peak-to-peak amplitude of >200 μV in 3 of 5 consecutive trials. The stimulus was performed with the subject applying 10% of the isometric MVC, this % was display on a screen to make it easier for the subject to keep the same amount of force during the stimulations. AMT will be defined to calculate the intensities that are going to be used to analyse Short Intracortical Inhibition (SICI). Condition stimulus was set at an intensity of the 80% of the AMT and 120% of the AMT for the test stimulus with a 3 ms interstimulus interval(ISI) (Berghuis et al. 2016; Kidgell et al. 2013; Rosenkranz et al. 2007). Ten stimuli were delivered for the test conditions and 10 for the normalization.

Finally, for the silent period (SP) subject had to follow a line set up at the 50% of the MVIC, then 5 stimuli were applied at the 120% of the AMT. In every condition stimuli, a randomised delay of 5-8 seconds was applied.

7.3. Transcranial Direct Current Stimulation

TDCS was applied during the intervention with a HDCStim (Magstim, Whitland, UK) current stimulator. Anode was placed over the hotspot of the non-dominant tibialis anterior on the motor cortex area (2-3cm posterior and 1-2 cm distal from the vertex to the right hemisphere) determine by TMS stimulation. The cathode was placed on the ipsilateral shoulder (Saucedo Marquez et al. 2013; Schambra et al. 2011). The electrodes were 5cmx5cm, both, the anode and the cathode. 2 mA were deliver to the subjects on the Stimulation conditions for 15 min, this was ramped up at the beginning over a period of 27s to reach the intensity and end of the training over a period of 27s, with a current density of 0.08 mA/cm^2, during the intervention procedure. Sham condition was given by ramping the stimulation up and down over 27 s at the beginning and end of the 15 min training. Researcher and Subjects were blind to the type of stimulation that was applied. In every single session, subjects were asked about any side effects that it may be caused by the tDCS. Two of the subjects had to drop out of the investigation due to an appearance of tinnitus symptoms.

7.4. Data analysis

Digital data analysis was performed off-line with Spike2 software (vDemo; CED). The software was programme to automatically control the stimulation offset and amplify the EMG signal.

MEP and SP. Peak-to-peak amplitudes of MEPs were calculated between the initial deflection of the EMG from the baseline (i.e., MEP onset) to the second crossing of the horizontal axis (i.e., MEP offset). Peak-to-peak amplitude were normalized to the Root Mean Square of 500m of the MIVC's EMG signal. The duration of the absolute SP was assessed by visual inspection from the MEP offset to the return of EMG activity. Variables from 10 TMS trials were averaged for each individual.

Motor task. The data collection was done with Spike 2 (v6.21), transferred to Matlab. In Matlab, the mean values for set and day were obtained through the automatic analyses of the difference between target and performance, being 0 the perfect performance and 8 the worst.

7.5. Statistical analysis.

Statistical analysis was performed using IBM SPSS statistics 23v (Chicago,IL, USA). Descriptive results are presented on TABLE 5 and. Normality of the distribution was assessed through Shapiro-Wilks test, due to the small number of the sample size. Difference between groups was analysed with an independent sample t-test for I/O curve, SICI and motor task day1, day 5 and day 6. Then, a paired-sample t-test was used to compared between day1, day 5 and day 6 for the same parameters mentioned before. For the normally distributed data parametric analysis was used and for the non-normally distributed non-parametric test were used . Significance was determined with $p<0.05$.

Even though in some of the intensities of the MEP and different conditions the distribution is not normal with $p<0.05$, the homogeneity of the covariance is normal in most of the cases that the distribution is not normal. Moreover, skewness is, in most of the cases over ±0.5 and even ±1.

TABLE 5. Mean and standard deviation of different Motor evoke potential at different intensities of the Input/output curve, SICI and corticospinal Silent Perriod (cSP) for SHAM and STIM.

	Day 1		Day 5		Day 6	
	SHAM	STIM	SHAM	STIM	SHAM	STIM
MEP 100%						
pre	65,35±42,13	52,74± 37,15	49,27± 28,12	53,33±30,07	32,25±6,94	54,76± 30,59
Post	62,82±51,24	95,43±47,89	50,07±24,10	76,22±46,16	42,05±12,68	56,42±27,85
MEP 110%						
pre	102,9±57,52	110,99 ±119,12	74,81±51,93	112,93±82,62	46,52 ±25,3	54,76±30,59
Post	94,01±54,51	155,80±108,25	73,92±23,67	120,67 ±77,06	68,69±34,35	115,14 ±55,22
MEP 120%						
pre	129,6±63,18	148,35±106,11	126,10±50,82	107,64±66,02	72,04±43,84	112,20±76,11
Post	126,67 ±98,62	153,27±104,15	117,29 ±50,04	138,90±79,33	89,26±48,95	139,96±74,80
MEP 130%						
pre	143,71±65,49	200,77±145,98	133,93±77,60	149,15±78,84	108,37±63,55	131,34±91,86
Post	134,82 ±72,26	179,42±116,97	112,77±53,89	155,76±89,99	102,11±40,15	158,69±99,14
MEP 140%						
pre	185,58±106,32	228,28 ±126,995	139,33 ±81,69	145,82±78,22	108,37 ±63,55	131,89±101,84
Post	157,92 ±84,81	179,41±116,97	133,14±66,22	169,81 ±122,45	94,03 ±42,07	139,90±79,88
SICI						
pre	78,46±42,99	130,15±68,65	73,49±33,54	82,93 ±29,21	66,18 ±30,51	91,15 ±41,71
Post	73,56 ±30,85	98,38 ±47,90	98,32±40,31	100,66±31,90	73,78±30,93	81,88±12,22
cSP						
pre	,0698 ±,0244	,0629±,0318	,0753± ,0438	,0715 ±,0324	,0862±,0416	,0674±,0325
Post	,0792 ±,0249	,0705 ±,0465	,0714±,0561	,0737±,0405	,0931±,0390	,0570±,0366

TABLE 6. Mean and Standard deviation for every day motor task on SHAM and STIM. The closest to 0 the better performance.

	Motor task	
	SHAM	STIM
Day 1	2,60 ±1,75	3,45±1,80
Day 2	1,77±1,24	2,41±,91
Day 3	3,02±1,29	2,08±1,31
Day 4	2,92±1,37	1,73±,94
Day 5	1,39 ±,91	3,01±1,92
Day 6	2,04±1,59	2,55±1,54

8. Results

8.1. Motor task

Figure 19 shows the error that the subject had in each day during the task performance. This error is calculated by the difference between the target and the task performed. The mean values are the average of every set performed during the day, being 0 a perfect performance.

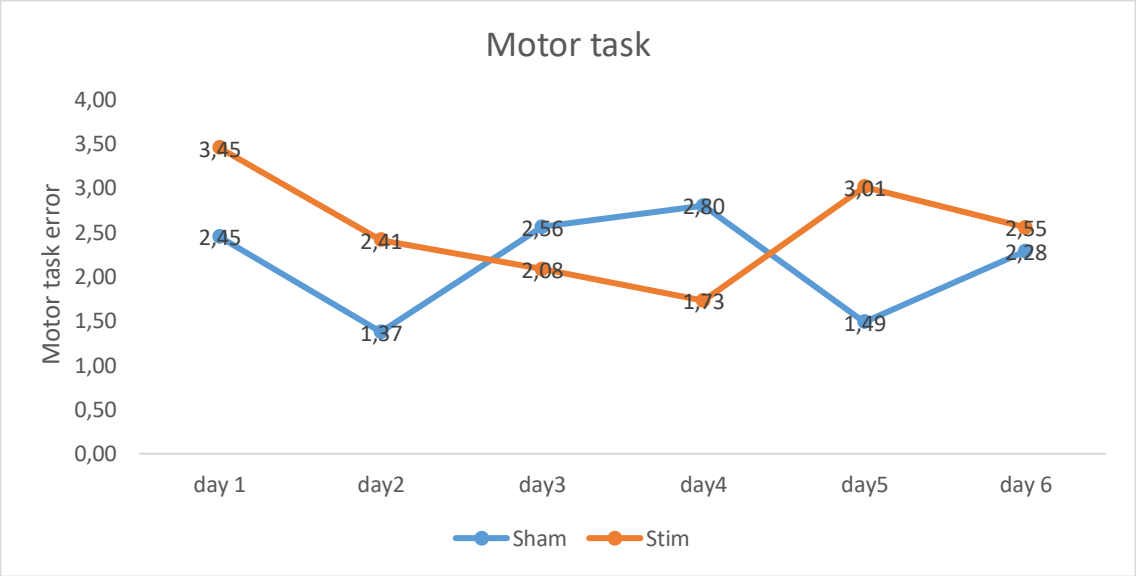

FIGURE 19. Mean values for motor task error. Orange is for STIM condition; blue is for SHAM condition.

For the motor task, there is no significant results for any of the conditions or any difference between days. P values were >0.05 within days and groups. As FIGURE 19 shows there is a big fluctuation on the motor task error

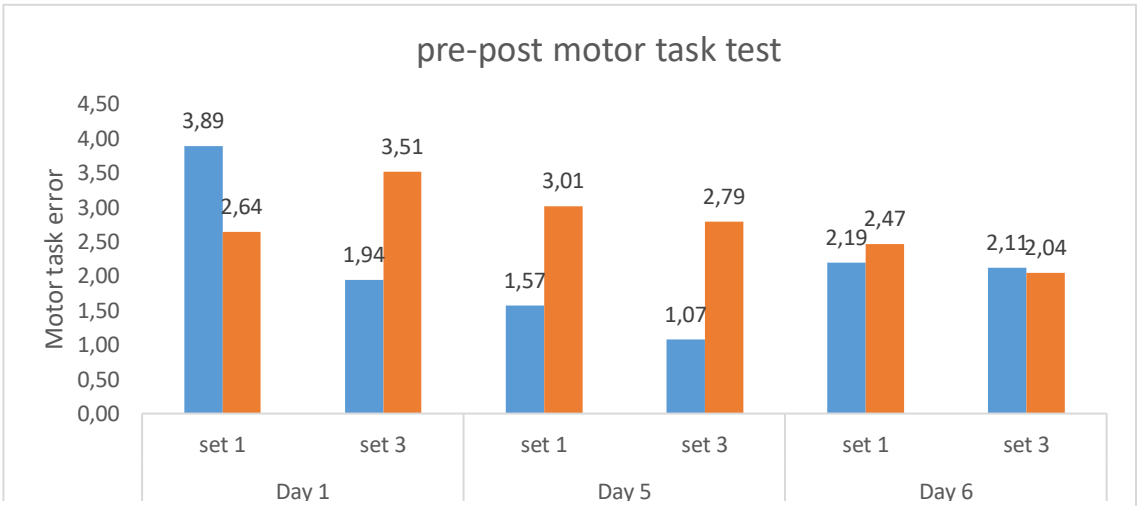

FIGURE 20. Mean values for motor task error pre-post testing day. Orange is for STIM condition; blue is for SHAM condition.

There is significant difference on the SHAM group on day one, as can be seen in FIGURE 20. The motor task error decrease from the set 1 to 3(P<0,05). However, there were not significant difference between groups or any other day and set.

8.2. Input/ Output curve, Short Intra-Cortical Inhibition and Silent Period

Motor threshold seems to change slightly between days and measurements, but there are not big changes in the resting motor threshold (Day 1: SHAM:46.8 ±14.86, STIM: 47.71 ±3.86; Day 5: SHAM: 48.8 ±15.48, STIM: 47.71 ±5.15; Day 6: SHAM: 48.4 ±12.36, STIM: 48 ±4.29) and either on the active motor threshold (Day 1: SHAM:47.6 ±18.12, STIM: 42 ±9.16; day 5: SHAM: 44 ±22.09, STIM: 41.43 ±7; Day 6: SHAM: 49.2 ±20.5, STIM: 40.5 ±6.16).

8.2.1. Input/output curve

There is not significance difference between different conditions and different intensities of rMT in any of the days and timing test. Either parametric and non-parametric analysis showed p>0.05, therefore no significance was found in any of the analysis that were carried out for the Input/output curve.

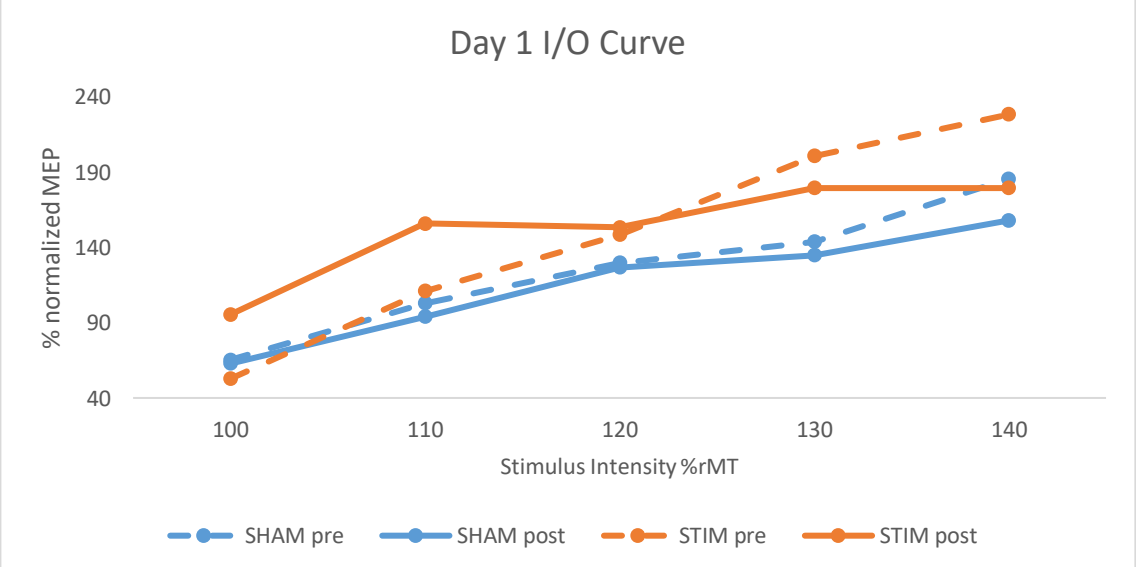

FIGURE 21. Mean values of Pre and Post I/O curve measurements day 1. Orange is for STIM condition; blue is for SHAM condition. dashed lines represent the pre-intervention and the solid line the post-intervention measurements

Moreover, Day 1 after the session, in the SHAM and STIM condition, the subject decrease the intensity of the MEP on the highest intensities of stimulation from the pre-intervention values. Although, the reduction on the STIM seems greater than in the SHAM condition. Moreover, there are significant difference between day 1-5 at 130% of MEP intensity. While on the day 5, looks like there is an improvement from the baseline in the STIM condition, while the SHAM still decreasing the intensity of the MEP and there is significant difference between post day1-5 130(p=0.045).

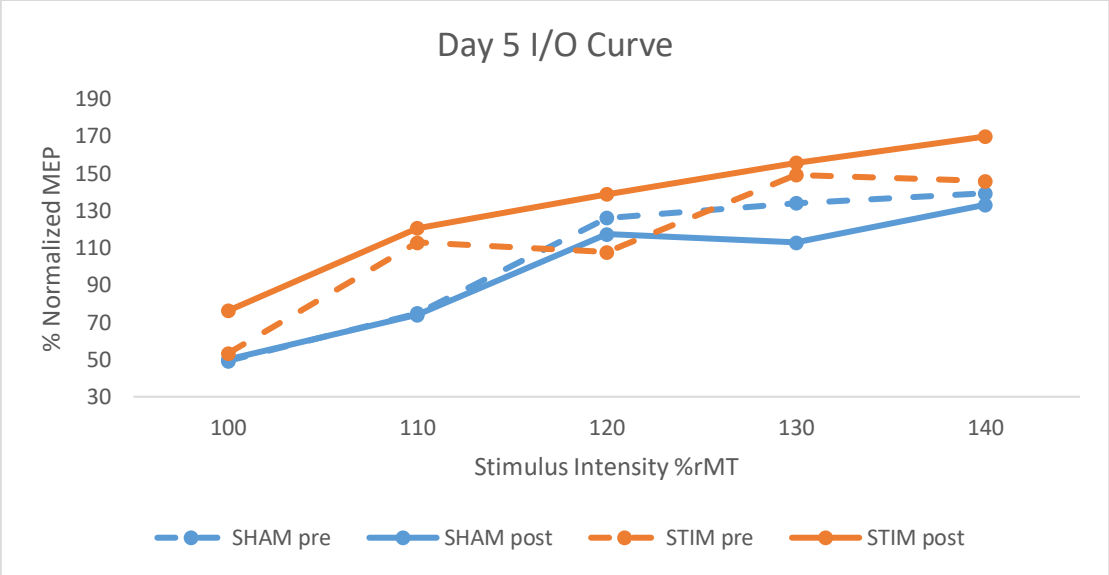

FIGURE 22. Mean values of Pre and Post I/O curve measurements day 5. Orange is for STIM condition; blue is for SHAM condition. dashed lines represent the pre-intervention and the solid line the post-intervention measurements.

Furthermore, in Figure 23, increase on the lower intensities of the rMT can be seen on day 6. There is significant difference between pre-post day 6 110% with a p=0.001. However, for the 120%, even though it looks a big change, there is not significant different for that intensity on the day p=0.061. Also, if there may not be as a greater improvement on the corticospinal excitability for the STIM condition at the higher intensities, there may be some at higher intensities for SHAM.

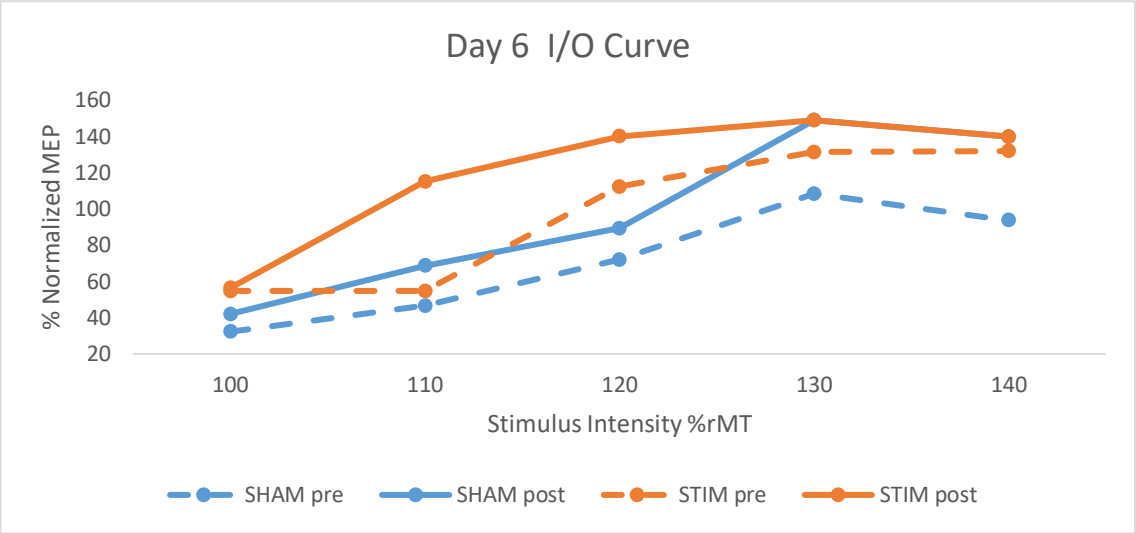

FIGURE 23.Mean values of Pre- Post I/O curve measurements day 1. Orange is for STIM condition; blue is for SHAM condition. dashed lines represent the pre-intervention and the solid line the post-intervention measurements

8.2.2. Short Intra-Cortical Inhibition

Significant differences from the short intracortical inhibition (SICI) results were not found between groups. Although, some significance was found between test days. There is a significant reduction of the MEP side from post-test day 5 to post-test day 6($p<0.05$). Meaning that even though, there is not significant difference between groups, there is a reduction on inhibition after a period of rest for both groups

FIGURE 24 shows a decrease on the intensity of the MEP from pre-to post, pointing, an increase on the inhibition for both groups. Nevertheless, SHAM condition changes are not as sharp as the STIM condition. Moreover, on day 5 there is an increase on inhibition for both groups in the pre-test, although it is greater for the STIM condition again. However, the inhibition reduced abruptly by the post measurements on day 5 for both conditions.

Furthermore, an increase of the inhibition for the SHAM condition can be appreciated, although for the STIM condition there is a small decrease from post- day 5 to pre- day 6. However, pre-post day 6, seems that STIM condition reduce, again the inhibition, while sham condition slightly increase the inhibition as FIGURE 24 shows.

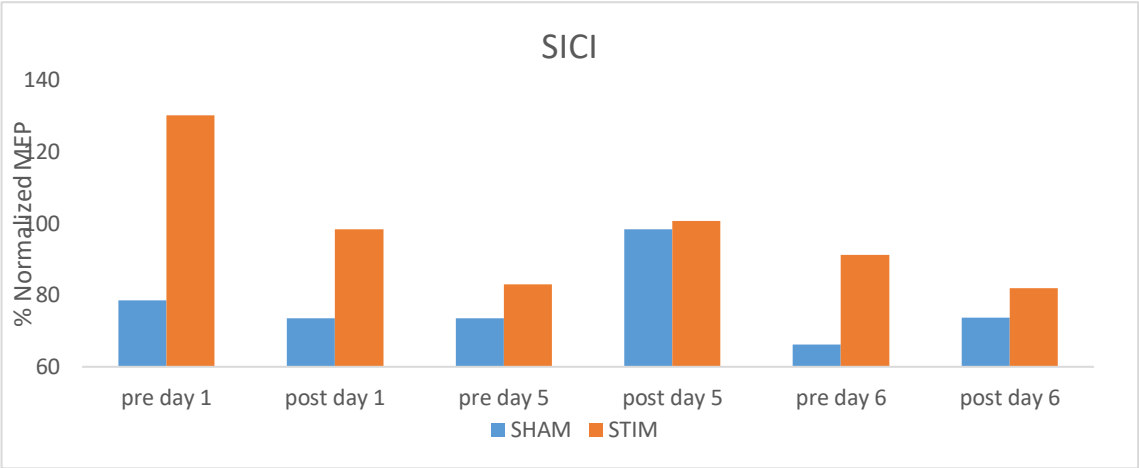

FIGURE 24. Mean values for different timeline testing. Orange is for STIM condition; blue is for SHAM condition.

8.2.3. Silent Period

For the silent period results, no significant results were found with the lowest value of significance found in the pre-post day 1($p=0.217$) and no difference between groups, with the lowest value on post day 6($p=0.132$).

9. Discussion

There has been a certain popularity on the properties of transcranial direct current stimulation(tDCS) and its effect on learning of a motor task and changes on the corticospinal excitability. There are different studies where they use different positions of the electrode, different intensities and timing. Therefore, this study was looking on what would be the effects of tDCS on a simple tibialis anterior isometric dorsiflexion motor task. Moreover, it looked on how its effects on motor learning and over the corticospinal excitability. Thus, an Anodal tDCS with unilateral positioning over the right motor cortex was applied over a period of 5 days and control whether its effect is maintained over a period of 8 days without being applied.

9.1. Transcranial direct current stimulation and motor learning

On the results, there is not significant difference between groups during online performance (during intervention) and during offline performance (after intervention), even though, there seems to be a better improvement on the error task for the sham condition. However, unlike this research, there has been shown that tDCS conditions enhance motor learning task in the upper and lower body not only during online performance. (Buch et al. 2017; Foerster, Dutta, Kuo, Paulus, & Nitsche 2018; Saucedo Marquez et al. 2013; Savic & Meier 2016; Veldman et al. 2016)

Maybe, the timing of the placement of the tDCS, either prior or during the motor task may have played a role during this research. However, Sriraman et al. (2014), found that tDCS prior to the practice did not improve motor learning as it did the one during, although there were not much differences on the retention test 24h after the intervention. On the same way, Stagg et al. (2009), point that tDCS prior to the practice will not have an increase in motor performance.

Nevertheless, one of the main differences in relation with the present study is the positioning of the tDCS, which may affect motor learning acquisition. In different studies, the unilateral position of the cathode has been on the contralateral side (Foerster et al. 2018; Shah et al. 2013; Tanaka et al. 2009). Although, when related to study, the cathode

has been placed on the ipsilateral shoulder side, whereas in this study, the cathode has been places in the contralateral side (Saucedo Marquez et al. 2013; Shah et al. 2013).

Nevertheless, the present study count with a small population for the online performance (n=13) and even smaller for the offline performance (n=12), with half of the participants in one group. Thus, although difference in the mean value can be appreciated, the dispersion of the data is too big. Therefore, more subjects are needed to be able to see any changes since the sample size was not consider prior to the study. However, this may not be the only reason. Task difficulty may have been an issue, for both groups, during training session. In this study, a movement were only Tibialis Anterior was used at 20-50 % of the maximal isometric voluntary contraction, while Shah et al. (2013) used a plantar-dorsiflexion task from 60-80% of the individual's maximum comfortable range of motion showing an increase on accuracy for the non-dominant leg.

Fatigue is another aspect to consider, although task was designed to avoid fatigue, different studies support that tDCS enhance endurance isometric contractions (Angius, Pageaux, Hopker, Marcora, & Mauger, 2016; Cogiamanian, Marceglia, Ardolino, Barbieri, & Priori 2007). Also, Justice, Mani, Pierpoint, & Enoka (2014), showed that the average fatigue on a continues isometric dorsiflexion contraction for adults was 15.5 minutes at 20% of the MVC. Although the intervention time was the same, intensity was different for the intervention protocol in this thesis (20-50% MIVC).

Moreover, this study task was performed with feedback immediate after the task, not during the task as others have done so far (Foerster et al. 2018; Shah et al. 2013). This experimental task provides a terminal feedback, where the result is present after the motor task execution, while most of the task present a concurrent feedback (Schambra et al. 2011; Shah et al. 2013; Sigrist, Rauter, Riener, & Wolf 2013). However, even though concurrent feedback may be better for retention and consolidation because it produces and external focus of the target, it also creates dependence on the subject (Sigrist et al. 2013). Moreover, terminal feedback seems to produce better acquisition on simple task and better results on the retention test (Sigrist et al. 2013). Therefore, terminal feedback should be used more often since it is useful for more complex task, that could be related with sports without being dependant of it.

Furthermore, Héroux, Loo, Taylor, & Gandevia (2017) found that many papers were underpowered and publications may be bias by the significant results. Therefore, more research with big size of subjects is needed to see the real effect of this device.

9.2. Changes in corticospinal excitability due to transcranial direct current stimulation application

The results show that tDCS did not have a consistent effect over the motor cortex to enhance the excitability over the Tibialis anterior area. Difference were not found either on the reduction of inhibition in the cortical pathway. Nevertheless, there has been many research about tDCS and there is a lot of things that could affect the effect of the device. (Ammann et al. 2016; Buch et al. 2017)

Although, current density has been tagged as one of the main reasons why a protocol does not work, in the present study the current density was 0.08 mA/cm^2, which is above the minimum to produces corticospinal changes (Nitsche & Doemkes 2007; Turi et al. 2014). However, it seems that there is not an agreement in which could be the best current density to increase corticospinal excitability. Nitsche & Paulus (2000) and Nitsche et al. (2003) suggested 0.0029mA/cm^2 as the optimal one. Still, other current densities: 0.004 mA/cm^2, 0.125 mA/cm^2; applied during motor learning have found changes in corticospinal excitability (Kidgell et al. 2013; Sriraman et al. 2014).

In the same line, Bastani & Jaberzadeh (2013) put into test 4 different current densities: 0.013 mA/cm^2, 0.029 mA/cm^2, 0.058 mA/cm^2 and 0.083 mA/cm^2. They found that the different current densities they tried, produced different changes in the corticospinal excitability of the subject. That these changes where attained for 30 minutes after the stimulation and the lowest current densities, produce greater changes on the corticospinal excitability. Thus, it seems that lower current densities may have a better effect (Bastani & Jaberzadeh 2013) than the one suggested as the beginning by the optimal one (Nitsche & Paulus 2000). This can also be cause by the electrode size, in which small electrodes are more focal than bigger ones (Bastani & Jaberzadeh 2013; Nitsche & Doemkes 2007).

Moreover, tDCS produce variability in the corticospinal excitability that may apply in this case (Horvath, Vogrin, Carter, Cook, & Forte 2016; López-Alonso, Fernández-del-Olmo, Costantini, Gonzalez-Henriquez, & Cheeran 2015). This variability can be cause not only after the first day of application (Bastani & Jaberzadeh 2013; López-Alonso et

al. 2015), but also, after 9 days of use (Horvath et al. 2016). Thus, subjects under stimulation conditions might response differently from one day to another, increasing or reducing its MEP intensity without a normal patter.

In the case of Horvath et al. (2016) study, this response might change the excitatory or inhibitory response from one session to another, modulating the MEP amplitude up to a 15% 30 minutes after session. Some explanations may be the session testing and the time between them, coil position, increase fatigue, intra-session adaptation, mood, etc. However, López-Alonso et al. (2015), which methods did not use a neuronavigator to place the coil, found that the most unreliable data was after 30 minutes of the tDCS procedure, for MEP amplitude and SICI measurements. Nevertheless, all the studies mentioned above are related with the arm muscles, whereas this study is focus on the lower limb muscles. Therefore, other cortical structures could have been involved as part of motor control and motor plan of the lower limbs (Kesar, Stinear, & Wolf 2018; Schmidt et al. 2018)

Moreover, during this experiment SICI was taken before cSP, which may modulate the response on the later one, making it shorter. This response may be due to the activation of the inhibitory intermediate neurons that are activated by the SICI measurements (Kojima et al. 2013). Also, consideration need to be taken on the first part of the Silent period (50-75 ms), where there is a contribution from the spinal inhibition, although the late part is supraspinal, with a presumably origin on the motor cortical area (Butler, Petersen, Herbert, Gandevia, & Taylor 2012; Ziemann 2013).

10. Conclusion

tDCS is a device that may enhance motor learning and modulate the corticospinal excitability. However, this thesis has not found any motor learning difference with the application of tDCS during 5 days of application or any retention benefits. However, we found that it may cause a great variability in the corticospinal excitability after its use. More research is needed to better understand the effect that tDCS can produce in motor learning and cortical excitability depending on the positioning, intensity, current density and timing.

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