THE EFFECT OF TRANSIENT VISUAL DEPRIVATION OF MOTOR CORTICAL EXCITABILITY AND MOTOR LEARNING

A Pilot Study

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ABSTRACT

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We devised an experimental protocol to test the hypothesis that if the performer poses attention on the proprioceptive signals, in the form of awareness of the body in space, this could increase the corticospinal excitability and the overall performance. 10 right-handed volunteers were randomly assigned to either a group performing a skill training blindfolded (BLIND) or in normal (NORM) condition. The intervention included a visuomotor task-related skill training lasting five days. Neurophysiological measurements included input/output relationship (IO Curve) of the motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI), both measured with the transcranial magnetic stimulator, from the right Biceps Brachii muscle.

The results showed no significant changes in any of the neurophysiological parameters, either between the groups or within the same group. However, the analysis revealed that in day 1, the BLIND group showed an increase in the corticospinal excitability compared with NORM. There was also a slight decrease in SICI for BLIND, and an increase in SICI for the NORM group. On day 5, there was a slight reduction in the corticospinal excitability for BLIND, coupled with a slight increase in SICI. For the NORM group, on day 1 there was a decrease in the corticospinal excitability, coupled with a decrease in the SICI as well. On day 5 the CSE increased, while the SICI decreased.

The performance, expressed as mean error between the trial and the target line, showed that the BLIND group performed better in each day, with a strong significant difference on day 1 (difference between the groups: 3.07 ± 1.17, $p < 0.01$). On the other hand, the rate of performance improvement was significantly greater for the NORM group, compared with the BLIND group (difference between D1 and D5 for the NORM group: 3.70 ± 1.59, $p < 0.01$).

It is concluded that a skill training performed blindfolded is undoubtedly beneficial for the performance, and that a modulation of the corticospinal excitability is indeed in place. Future study will be needed to clarify the exact mechanisms behind this modulation.

Keywords: Transient Visual Deprivation; Transcranial Magnetic Stimulation; Motor Skill Learning; Visuomotor Practice.

TABLE OF CONTENTS

INTRODUCTION

During a match in the 1991 NBA championship, the great basketball player Michael Jordan scored a free throw with his eyes closed (figure1). That was taken by everybody as remarkable move by Jordan, and contributed to the perception of the player as the greatest basketball player of all time. In movement science, a free throw can be considered as a goal-directed movement, that is a movement controlled purposefully towards a certain goal or target, in this case the basket. The fact that Michael Jordan scored even though the visual stimuli, which direct the movement in a phenomenon called "hand-eye coordination", were not available it is a striking result, and an extremely interesting phenomenon to study.

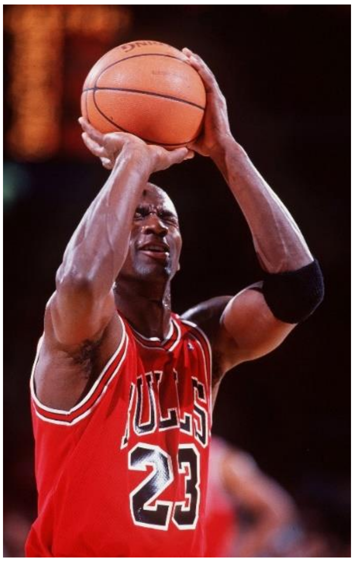


Figure 1 – Michael Jordan shooting a basketball free throw with his eye closed. Image from sneakerhistory.com

In practice, performing a training blindfolded is a common concept. For example, in basketball, specifically designed glasses do not allow the player to see the ball, and high level athletes use the blindfold to perform simple exercises in order to improve their efficiency (or at least this is the goal). Surprisingly, however, no scientific reference was found specifically aiming to model the use of a blindfold (or any other way to induce transient visual deprivation) in the training process. Therefore, questions arise on the validity of the method. The concept of the blindfolded training might seem a simple one:

the performer does not use the visual stimuli, and therefore it uses the remain senses to perform the activities, just like a blind subject. However, the scenario is more complex. Indeed, the withdrawing of vision is in general thought to be associated with an increase of attention posed on proprioception. This brings forward another problem, that is which sense has the dominance between the vision and the proprioception, or any other “sense”. In order to properly understand the concept of the blindfolded training, it is important to answer to several important question.

First of all, it is fundamental to understand how the movement is produced and controlled. Although it might seem rather general, this question includes several concept and problems that are fundamental for the validity of the blindfolded training. For example, what is the role of the subcortical levels in the production and controlling of movement. What is the role of the afference in the movement control: Is it just to react to environmental stimuli, or it is primarily involved in the control of movement?

The second, and maybe most important question is posed by the performer when the blindfold is applied is "where am I?". In other words, how can the subjects know where their body is in the environment if they can’t see? This is done by a multitude of senses acting together, and the so-called “Kinaesthetic sense”, which has a major role in this phenomenon. In addition, several authors have investigated the dominance of the vision and proprioception in the control of the movement, and found different roles of the vision and proprioception in different aspects of movement production.

Another important (and obvious) question to ask is "how is a skill learned?". This is a particular important question that coaches should ask themselves constantly (although rarely this happen). Indeed, in order to understand how excellence in performance is achieved by the top-level athletes, it is fundamental to understand “how do we learn”. Motor learning can be analysed at various levels of analysis, from the behavioural to the neuroscientific principles behind the improvement of the performance. In each level of analysis, the process of learning a new skill has always been defined as a multistage process.

The present thesis, therefore, is an attempt to answer to those questions, as well as to provide scientific data and considerations on the use of the blindfold in motor skill learning.

1. HOW IS MOVEMENT PRODUCED?

1.1 – From Neuromechanics to Motor Control

The great scientist and philosopher Sir Charles Sherrington once said "*The essential service of muscle to life is, quickly and reversibly, to shorten so as to pull. Its shortening is called 'contraction'. The importance of muscular contraction to us can be stated by saying that all man can do is to move things, and his muscular contraction is his sole means thereto"* (Sherrington 1951, pp.116).

From this significant quote, we understand that movement is produced by the muscles. Although this is absolutely true, the reality is more complex. Figure 2 shows a schematic view of how movement is produced. The body features several structure deputed to control certain aspects of movement production. One of those structures is the muscle itself. In fact, inside the muscle there are the muscle spindles that control and detect the muscle length and contraction velocity. Furthermore, an additional structure of control of the movement reside in the tendon, the Golgi tendon organs (GTO) which, on the opposite, signal about the force of contraction (Enoka 2015, pp. 254-255). From the muscle spindles and the GTO (as well as other structures), the involuntary movements arise. These features will be exposed in chapter 2.

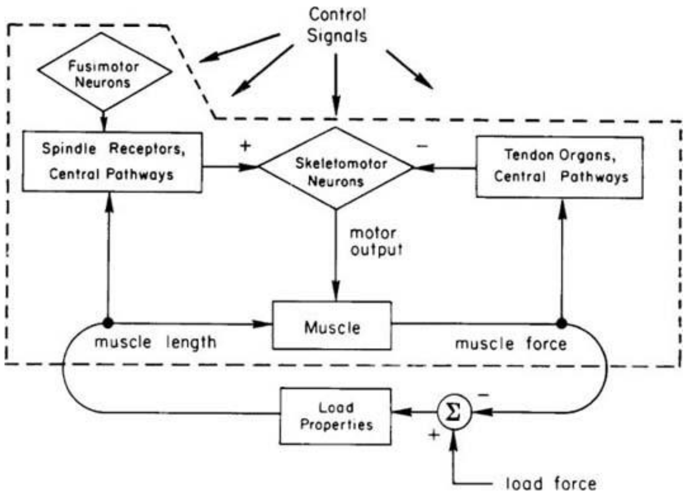


FIGURE 2 - A schematic representation of the movement control centres. From (Houk & Rymer 1981).

So after all, although complicated, we have pretty good idea of how movement is produced. However, another important question to ask when thinking about movement production is how movement is controlled. In other words, what type of information does the neuron carries when it fires? This is a fundamental question in neuroscience.

Today we know that the CNS is organized in a specific and efficient way. Briefly, the human body is featured with a series of sensors that detect changes in the environment and within the body itself. These information travel through ascending pathways, mostly in the spinal cord and project to the primary sensory cortices which deal with the physical nature of the stimulus. From there, the information goes through higher-order sensory cortices that deal with the reconstruction of the meaning of the sensory information. After that, the information goes to the prefrontal cortices, which deal with the cognitive meaning of the information, and from there, the movement pathways begin. The cortical control of movement follows the opposite pathway compared with the sensation. In fact the information passes first through the high-order motor areas, and terminates to the primary motor cortex (M1), where the corticospinal neurons leave the brain to project to the α-MN in the spinal cord (Kandel et al. 2013, pp. 392-409).

The question of what type of information are carried when the neurons fire is a major question in neuroscience. Faux (2002), for example, argued that neuroimaging, which allow us to assess the brain areas that are activated by a certain stimulus or associated with neural processing, is nothing more than a “new phrenology”. If we put this argument into the context of movement and perception-action coupling (PAC), we see that although we have modelled the movement production, yet there is not a consensus on what kind of information are carried from the corticospinal neurons to the α-MN, and from the α-MN to the muscles. In order to have an idea of what “language” the neuron uses when fires, certain models in neuroscience and motor control have been advanced. Some models suggest that the α-MN carries a motor command with a full specification of the movement (Kawato 1999), other perspectives suggest that the α-MN carries only salient aspects of the movement, and the rest of the movement is controlled subcortically, given the interaction of the body with the environment (Feldman 2015, pp. 22-29; Scholz & Schöner 1999).

1.2 – How is Movement Controlled? Introductory Elements of Motor Control

In order to get as close as possible to the answer to the question of how the brain controls the movement, it is important to note that the brain faces a big problem when controlling the movement production, that is the body, at any level of analysis, has more ways to produce the movement than absolutely necessary for any given task (Latash 2012b). This principle is known as redundancy problem and it is one of the oldest problems in motor control and movement science. It refers to the fact that for any given multi-joint task, the combination of the joint configurations that solve the task is higher, compared to the task requirements. This feature of the motor system produces motor variability as a result (Latash 2012b).

The oldest example comes from Nikolai Bernstein and his seminal work on blacksmiths. Using video analysis, he was the first to show that while the tip of the hammer was constant through the trials, the joint configuration was variable (figure 3). In other terms, he was the first to suggest that the brain controlled some aspects of the movement, while other aspects of the movement were apparently not controlled (Latash 2012a, pp 15-16). For a general discussion on the Bernstein's idea of motor control, the reader is advised to read the good review by Bongaardt & Meijer (2000).

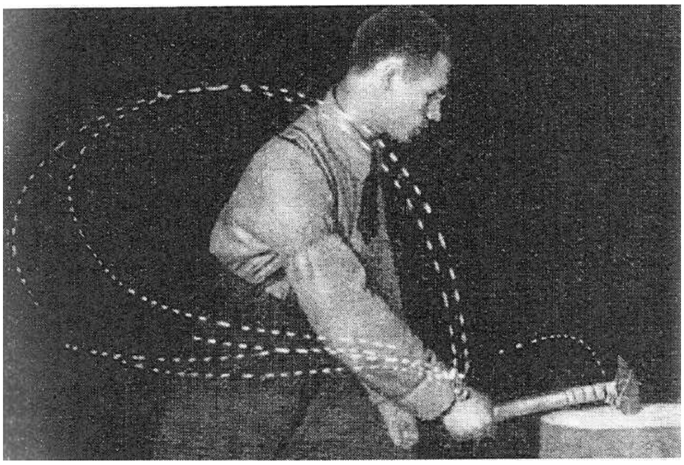


FIGURE 3 - N. Bernstein was the first to recognize that the brain controls salient aspect of the movement, leaving other variables not controlled. In this picture it is possible to appreciate that while the tip of the hammer remains controlled, the hammer smith shows inter-trial variability (Image from the web page: https://en.wikipedia.org/wiki/Nikolai_Bernstein).

In 1995, Schoner (1995) posed the question of what does it mean that the CNS controls a variable. He defined the controlled variables as those measures of behaviour that are stable against perturbations. Given the obvious fact that the environment in which the vast majority of human movements are produced is highly unpredictable, stability can be considered as fundamental requirement of the CNS. Within this context, Stability can be defined as the capacity of the system to return to a given state after a phasic perturbation, that brought the system away from that state (Scholz & Schöner 1999). The fact that the CNS controls a certain variable poses the problem of which variables are not controlled by the CNS.

Schoner (1995) used a good example to clarify the difference between the controlled and uncontrolled variables using a reaching movement towards a target. In order to simplify the conceptualization, he assumed that the CNS cares just on the movement pathway of the end-effector (EE) being straight. If a perturbation is applied to the EE, the high degrees of freedom (DOF) of the upper limb can compensate for the perturbation, leaving the EE position invariant. Therefore, although the EE position is a controlled variable, the limb configurations are not, since they do not have the requirement of stability in order to be called "controlled" (Schoner 1995).

Virtually all everyday movements are performed facing various perturbations at various levels, i.e. external or internal. Furthermore, the internal perturbations can be mechanical or neural (Scholz & Schöner 1999). Thus, the CNS faces constantly a high level of uncertainty when producing even the simplest movement. From a theoretical point of view (the practical application will be exposed in the next sections), the idea of the CNS controlling certain variables that bring about stability to the movement production, while leaving other variables "uncontrolled", ensures an effective strategy to ensure an optimal performance even in highly unpredictable environment (Schoner 1995).

1.3 – Models of Motor Control and their Influence on Perception-Action Coupling.

As suggested earlier, throughout the years, various models of motor control have been advanced. Although the explanation of these models are beyond the purpose of this review, it is useful to note that models are common in science and that, after all, science itself is a process of modelling the reality (Rosenblueth & Wiener 1945). Although it might seem a philosophical point, the models have usually practical application in the discussions of the researches, where the results of experiments are discussed and put into context. Different models suggest different contribution of the various components that are included in the model. Since the idea of this thesis project relies on the concepts of the perception-action coupling, it is useful to briefly introduce these models. For the reader who would like to gain more information about those models, great books about motor control like Feldman (2015), Kandel et al. (2013), and Latash (2012a) can provide an exhausting overview and serve as a starting point to dig into the topic.

The role of proprioception in movement production has been modelled according to various perspective. For example, one of the most influential view is that the movement is produced mainly by internal models (Kawato 1999) and motor programs (Schmidt & Wrisberg 2008, pp. 107-110), that is the brain pre-determine patterns of EMG, kinetics and kinematics variables using computations. In this view the proprioceptive information have a marginal role in the movement execution and, to remedy to the following consequence of the posture-movement paradox (reviewed in Ostry & Feldman 2003), it is suggested that the sensory information coming from the periphery is subtracted from the assumed sensory integration (Proske 2005), in a phenomenon called “efference copy”. See chapter 2 for a better explanation.

Another view, the equilibrium-point hypothesis (Feldman 2011), suggests that not only proprioceptive information are important for the control of movement, but it brings this concept further, suggesting that they actually participate to the production of movement. According to this perspective, movement is the transition between the desired posture (or referent) defined by the CNS, and the actual position, which information come from the proprioceptor sensors. Therefore, the proprioceptive signals are fundamental for movement production, because they define the muscle activity that is required to minimize the difference between the referent and the actual position (Feldman 2011).

2. WHERE AM I?

2.1 – The Concept of Proprioception

In his famous book "*The man who mistook his wife for a hat*", The neurologist Oliver Sacks (Sacks 1985, pp 69-83) reported the true story of Christine, a twenty-seven years old lady who lost the capacity to sense her body, the proprioception. Throughout the fascinating story of the partial recovery of Christine's proprioception, even a reader without any knowledge in movement science can understand how devastating and invalidating the loss of the capacity to sense the own body is.

The concept of proprioception is explained really well again by Sherrington, who defined it in the following way: "*In muscle receptivity, we see the body itself acting as stimulus to its own receptors – The proprioceptors*" (Proske 2005). Different "senses" are included in the more general term of proprioception. Proske (2005) suggests that 4 classes are conventionally accepted. The kinaesthetic sense, generated by the muscle spindles, the sense of tension, generated by the Golgi tendon organs (GTO), the sense of balance, generated by the vestibular system, and the sense of effort, that is thought to be generated by the combination of various senses from the CNS.

Although all senses participate to the overall perception of the body, for the sake of this discussion, only the kinaesthetic sense will be exposed. In addition, other aspects of perception (like vision) will be linked together. The purpose is to create a wide, evidence-based picture about the ability of the system to sense its own posture and movement, and to join this type of information with the ones coming from the outside world, in order to produce movement.

2.2 – Kinaesthesia

2.2.1 – The Kinaesthetic Sensor

As suggested before, the kinaesthetic sense is defined as the perception of the body position and movement. The sensors that are responsible for this sense are the muscle spindles, which are fusiform sensory receptors encapsulated within the muscle. The muscle spindle is composed of a bunch of special fibres (intrafusal fibres) oriented in parallel with the rest of the muscle fibres (extrafusal fibres). These intrafusal fibres are different in composition and anatomy compared with the extrafusal ones. In fact, these fibres have contractile properties only in their polar regions, while the centres of these fibres have sensory purposes. The centre of the muscle spindles, in fact, is the “house” of the sensory afferent neurons, namely type Ia and II. The Ia afference is thicker compared with the type II, and it is sensitive to the velocity of the stretch, while the type II afferent neuron has a slower conduction velocity, and its discharge provide information about the steady-state of the muscle length. In this purpose, the Ia fibres help the type II fibres.(Banks 2015)

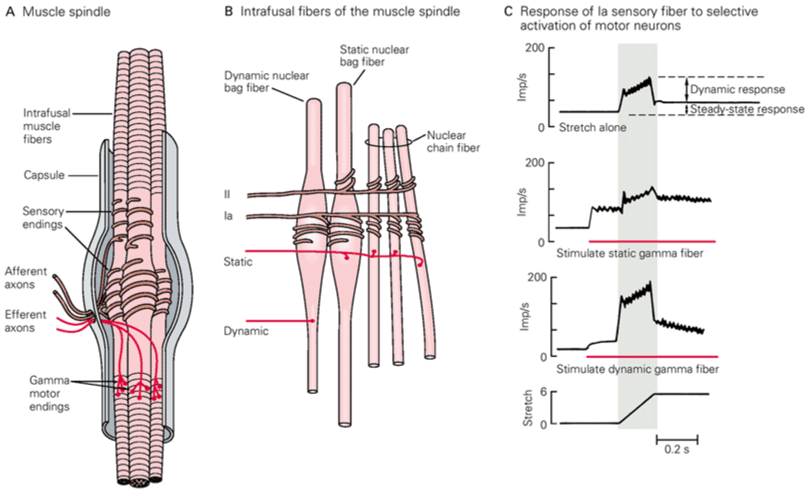


FIGURE 4 – A schematic representation of the anatomy of the muscle spindle, as whole (A) and the innervation of the intrafusal fibres (B). (C) shows the neural response of the Ia fibre with the muscle stretch alone, and with stimulation of the static and dynamics gamma fibre (Kandel et al. 2013, pp 795).

Muscle spindles (MS) are under the direct control of the CSN (figure 4). In order to properly do their job even during a contraction, the polar regions of the muscle spindles are innervated by the γ-motoneurons (γ-MN), a small motoneurons similar to the α-motoneurons (α-MN). When the γ-MN fires, the polar region is activated, thus adjusting the sensitivity of the muscle spindles to the current length of the muscle (Kandel et al. 2013, pp. 802-804). In order to properly exert its function, the γ-MN receive neural signal at the same time of the α-MN, in the so-called “alpha-gamma co-activation”. This makes sure that both the muscle has the descending signal to shorten, and the muscle spindle can adapt its sensitivity to this descending signal (Kandel et al. 2013, pp. 802-804)

The afferent fibres, Ia and II, project to the spinal cord. It is interesting to note that there is variability in the projection of the afference to the spinal cord. Jankowska (2015) suggested that the projection of the MS afferent fibres seems to have a random distribution. However, despite this apparent randomness, the afferent fibres seem to project to five specific areas of the spinal cord within the motor nuclei. These five areas correspond to the location of five main population of neurons, that is motoneurons, Ia inhibitory interneurons, ventral spinocerebellar tract neurons, intermediate zone interneurons and dorsal spinocerebellar tract neurons. In addition, Jankowska (2015) suggested that the afference converge on several population of spinal neurons following a specific pattern, ensuring a purposefully interaction. In fact, as figure 2 shows, the muscle spindle projects back to the motorneurone. The summation of the peripheral signals, mediated by the proprioceptors, and the descending commands from the central structures defines the membrane potential of the α-MN, making it fire (Feldman 2011).

2.2.2 – The Kinaesthetic Problem

Today the general consensus, supported by exhaustive literature, is that the muscle spindles are concerned with signalling the changes in the muscle length and position sense. However, multiple authors have suggested that the muscle spindles are potentially ambiguous in this type of sensation. One problem comes from the fact that activity can be generated by either intrafusal (via a γ-MN activation) or extrafusal contraction (Proske 2005). In addition, the response of the passive muscle spindle is dependent of the history of contraction, a phenomenon called thixotrophy (Proske et al. 2014). An additional problem with the muscle spindles as position sensors is that the firing pattern of the muscle spindle is ambiguous with respect to the type of contraction. For example, during an isometric contraction, the limb is perceived as non-moving, while the muscle is shortening (Hulliger et al. 1982).

Taken all together, these problems raise a question "*How is it that the position sense remain accurate, despite the ambiguity in the activation of the muscle spindles?*" (Feldman 2008). Feldman (2009) suggested the term "kinaesthesia problem" to refer to this ambiguity. During the past years, models to solve this problem have been suggested. For example, one of the most frequently cited, as suggested before, is the "efference copy" concepts. Proske (2005) reported that this principle could be applied to the kinaesthetic sense in way that "*Any fusimotor-evoked activity would be subtracted from the total* *spindle signal, effectively leaving only the passive component of the response to be registered as a sensation*". It is interesting to note, however, that the same author, right after, suggests that "*It is not clear how such a system would work in practice, given the variable relationship between motor output and reafferent signal*" (Proske 2005).

Another way to solve the kinaesthetic problem comes again from the equilibrium point hypothesis. Feldman (2009) suggests that the afferent signals should not be conceived as carrying information about the absolute body position, but rather on the deviation of the body (Q) from its referent configuration (R). The difference between the referent and the deviation of the actual configuration, give rise to the kinaesthetic sense. This theoretical mechanism has been named "Position Sense Rule" (Feldman 2015; pp. 195-197).

2.3 – Vision

Doubtlessly, the visual system is one of the most important features of the body. By seeing things, we can perceive the external world, and we can direct our actions with an extreme precision to targets, even in presence of obstacles. (Goodale 2011)

A schematic representation of the visual pathway is depicted in figure 5. It begins from the eyes, which act as collector of information from the external world. The retina is the structure deputed to the capturing of those information. The retina is formed by multiple photoceptors, which capture the light, organized into two hemiretinae, the left and right. The photoceptors have synaptic links with the ganglion cells. The axons generated from the ganglion cells form the optic nerve. Each optic nerve is made by neuron from the nasal and the temporal side of the same eye. Each hemiretina captures information from the opposite side: the temporal side of the left eye will capture information about the right side of the visual field, whereas the nasal side of the left eye will capture information about the left side of the visual field. The opposite will happen for the right eye. (Kandel et al. 2013, pp. 556-576)

The nasal and temporal neurons of each eye run together until they reach the optic chiasm. Here the optic nerve splits into two, with the projections from the temporal side of the eye carrying on running in the ipsilateral path, while the nasal projections cross to the contro-lateral hemisphere. This makes sure that each hemisphere carries the same information of the visual field. The new joined ipsi- and contro-lateral projections form the optic tract, which extend until the lateral geniculate nucleus (LGN), located into the thalamus. Each LGN projects to the primary visual cortex (V1) through the optic radiation. (Kandel et al. 2013, pp. 556-576)

As said before, vision is really important for perceiving the external world and interacts with it. However, multiple researches have suggested an important implication in action as well. Goodale (2011) suggested that the visual system began as system for distal control of actions, rather than as a system for perceiving the external world. Regardless of why the visual system developed, it is doubtless that the visual system is important for both visual perception and control of actions.

The processes of visual perception and control of action do not finish at the level of V1. Indeed, from V1, the information travel into 2 pathways: a ventral stream (VS), directed to the infero-temporal cortex, and a dorsal stream (DS), projecting to the posterior parietal cortex (figure 5, Goodale 2011).

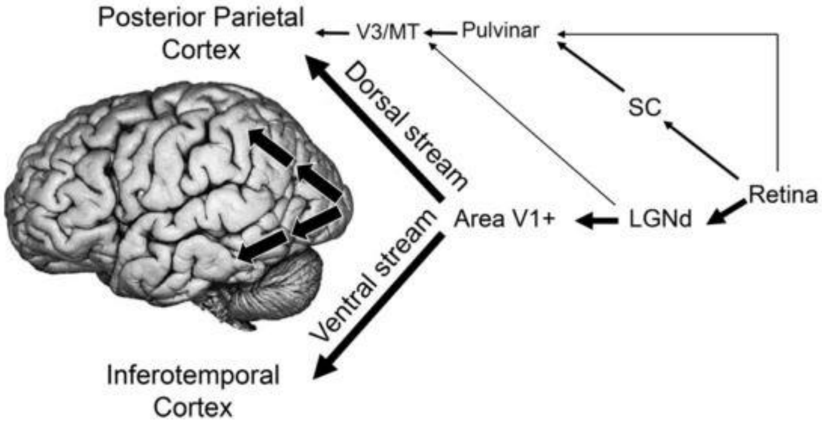


FIGURE 5 – A schematic representation of the visual pathway. The visual information leaves V1, and follows two paths, the dorsal and the ventral stream. Those two paths deal with different aspect of the information. From Goodale (2011).

The role of these two pathways, have been debated in the past years. In the original formulation of the dual stream framework, done by Mishkin et al. (1983), the VS was suggested to have a role in the identification and recognition of the objects, that is dealing with the *"what"* information. The DS, instead was suggested to have a major role in the localization of the objects, that is dealing with the *"where"* information. However, various researches have shown that this dualism does not exactly reflect the reality of the processes, and needed a re-interpretation (Goodale 2011).

The re-interpretation of the dualism in the pathway of the information from Goodale & Milner (1992), proposed that VS and DS reflected a different purpose of information processing. In fact, from their point of view, the VS has a major role in perception, providing the foundation in the off-line control of the action, whereas the DS, provide on-line control of action, that is generate skilled actions. In contrast to the model by Mishkin et al. (1983), the spatial and structural information of the percept are processed in both streams, but for different purposes (Goodale 2011).

2.4 – The Dualism Proprioception-Vision in Controlling the Movement Planning and Production.

Movement is always produced in an environment that changes with time. Most of the time, when the environment changes, it produces some stimuli that, according to the sensitivity of the sensors, are detected and processed. Those sensory stimuli have different physical nature. Conventionally, it is usually reported that our capacity to detect changes in the environment can be summed up in five senses: hearing, vision, touch, smell and taste. Proprioception can be considered as sixth sense. Although all those senses are fundamental for the survival of the human kind, and their loss creates a permanent damage in the way the person lives, (Merabet & Pascual-Leone 2010) for the sake of this thesis, only the two relevant senses to the main research question will be analysed.

Vision and proprioception deals with very different aspect of the sensation that have been exposed earlier in the text. Both senses, however, are important for the planning and the control of the movement. The information they provide are different in their nature. Vision deals with the detection of the outside environment, while proprioception deals with the signals coming from the own body (Sarlegna & Sainburg 2009). Thus, a question comes up naturally, that is which one has the dominance. This question has been studied extensively from various perspective and braches of science. For example, Touzalin-Chretien et al. (2010) used neuroimaging techniques (via event-related potentials), while others have used optical illusion (Mon-Williams et al. 1997), or covering of the visual field while the movement was performed (Brown et al. 2003).

Mon-Williams et al. (1997) used a Prism and a LED system to study to what extend the vision can alter the kinaesthetic sense. Their results are interesting, and surely add something to this discussion. Their results suggest that vision can produce a kinaesthetic illusion if the two information are not the same. However, this kinaesthetic illusion was produced only in a well-lighted room. In dark conditions, the proprioception had the dominance in the perception of the limb. In addition, they reported that in dark conditions the subjects felt that the LED (that was attached to their hand) was “detached from their fingers”. Producing therefore the opposite illusion. They concluded that although vision may have the dominance over proprioception, this dominance is reversed when the visual stimuli are poor, like in the case of a dark room.

Sarlegna & Sainburg (2009) suggested that vision and proprioception have different roles in the planning of movements. Multiple experiment, for example, have suggested that proprioception provides important information during the planning of goal-directed movements (Polit & Bizzi 1979). On the other hand, other experimental results suggest that vision has a crucial role in the definition of the spatial features of the movement. For example, Brown et al. (2003) designed an experiment to investigate the source of limb position drift when the visual feedback was removed during the movement. In addition, they were interested whether the position drift affected the movement production. They showed that while movement-to-movement position drift remained constant over time, the overall drift increased, and reached a plateau towards the middle block of 70 movement performed without the visual information. Using inverse and forward dynamics simulations they showed that the torques changed movement-to-movement in order to maintain the position. However, these changes contribute to the overall position drift. These results are paradoxal: It seems from these results that the proprioceptive information contributed to the movement trajectory control, but not in the maintenance of the spatial limb position. Therefore, Brown et al. (2003) suggested that the limb position and movement are controlled independently. This conclusion supports the idea of a hierarchical organization of the movement.

The fact that Brown et al. (2003), along with several other authors, have suggested that vision has the primacy in the planning of the movement could be expected. In fact, it has to be noted that living systems live in a highly unpredictable environment. Therefore, the capacity to plan and adapt our action according to the environmental conditions is logical. However, it seems that this primacy can be reversed in case the stimulus is transiently absent, like the case of Mon-Williams et al. (1997) or permanently absent, like the case of traumatic loss of sight (Gaunet & Rossetti 2006). This suggests that vision and proprioception work together in the planning of movement, although providing different type of information. Sarlegna & Sainburg (2009) suggested that the role of vision is to define the spatial feature of the movement, while proprioception provide information for the fulfilment of the movement. This suggestion is in accordance with the idea of hierarchical control of the movement, where the outcome is not defined by single level of control, but it is distributed between multiple areas and level of control (Feldman 2015, pp. 13-32).

It is interesting to note that the dichotomy between vision and proprioception has been studied in motor learning as well. Specifically, Proteau and his colleagues (Proteau et al. 1992; Proteau et al. 1998; Tremblay & Proteau 1998) suggested that the learning process is specific to the source of information available during the process. This hypothesis, developed in a series of studies that aimed to study the transfer of a new learned skill in different environmental stimuli, suggests that the learning process in based on both changes in the motor planning and error detection, and that the mechanisms of error detection are specific to the sensory stimuli in which the skill has been learned.

2.5 – Sensorimotor Integration and Proprioceptive Training; Is there any Difference?

A considerable amount of researches (reviewed in Ashton-Miller et al. 2001) have investigated the capacity of various interventions to improve the proprioceptive sense. The muscle spindle information does not stop in the spinal cord, but carries on in the dorsal column of the spinal cord, that is it ascends to the central areas. This is an important concept to point out since multiple authors have suggested that the proprioception can be improved (Ashton-Miller et al. 2001). Strictly speaking, an improvement in the proprioception with the training or with a treatment would mean that the actual muscle spindle would improve its ability to detect the changes in the muscle length, as well as an improvements in other receptors (Ashton-Miller et al. 2001). However, another way to improve the proprioceptive function could be archived by a better integration of the sensory information at cortical (Rosenkranz & Rothwell 2012) and spinal levels (Wolpaw 2007).

A series of studies from Rosenkranz and colleagues (Rosenkranz et al. 2003, 2007; Rosenkranz & Rothwell 2004, 2006, 2012) have shown interesting modulation of the corticospinal excitability (using TMS) by the proprioceptive information coming from the muscle spindles. All those studies have used peripheral muscle vibration, which activate the muscle spindle (Roll et al. 1989). Using this technique, they were able to show that low-amplitude muscle vibration increases the motor-evoked potential (MEP) of the vibrated muscle while, at the same time, decreasing the short-interval intracortical inhibition (SICI) (Rosenkranz & Rothwell 2003). In addition, they have shown that the Long-Interval Intracortical Inhibition (LICI) had opposite effect than SICI, that is, it was increased in the vibrated muscle, while decreased in the non-vibrated muscle (Rosenkranz & Rothwell 2003). They called this changes in excitability baseline "*sensorimotor organization*" (figure 6).

The term "proprioceptive training" is, therefore, a potentially ambiguous concept, as it does not define which component of the proprioceptive sense it aims to improve (Ashton-Miller et al. 2001). The term is supposed to indicate the improvement of the proprioceptive system, but it is ambiguous on which aspect of proprioception this training addresses. In fact since proprioception can be conscious (ability to detect the changes in muscle length) and unconscious (sensorimotor integration), it is hard to say if a specific evaluation and the following training specifically engage the conscious or the unconscious aspect of the proprioception (Aman et al. 2015).

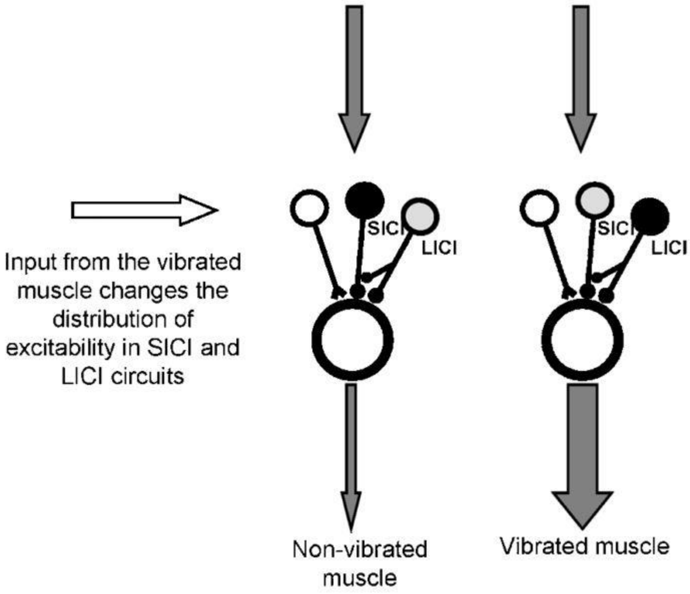


FIGURE 6 – A schematic representation of the mechanisms of the sensorimotor organization from (Rosenkranz & Rothwell 2003). The motorneurone (big circle) receives three inputs, the white neuron brings an excitatory input, while the SICI and the LICI are responsible for the inhibitory inputs. When the neuron is grey, the neuron's inhibitory power is decrease (that is the inhibitory neuron is less excitable), while the black colour represents an increase in the excitability of the neuron. As it is possible to appreciate in this image, muscle vibration changes the pattern of firing of the inhibitory neurons, which in turn has an influence on the output (grey arrow)

During the past years, the concept of proprioceptive training has been used by several author in researches as well as practical contexts. Most of the researches have been conducted on balance trainings (Aman et al. 2015 for a review of the literature). However, from previous researches on sensorimotor integration, it can be argued that any form of motor learning (which will be discussed in the next chapter) could be considered as proprioceptive training. This because motor learning has been associated with a reorganization and changes in the sensory system (Ostry et al. 2010), as well as motor system (see chapter 3). An interesting definition of proprioceptive training comes from Aman et al. (2015), which defines it as "*an intervention that targets the improvement of the proprioceptive function. It focuses on the use of somatosensory signals such as proprioceptive or tactile afferents in the absence of information from other modalities such as vision*".

The link between proprioceptive integration and motor learning has been provided by Rosenkranz & Rothwell (2012). They were interested in studying the role of attention to the proprioceptive signals during a motor learning task. The setup was similar to their previous studies (Rosenkranz & Rothwell 2003, 2004). However, they introduced two sensory attentional tasks, one designed to increase the level of sensory attention (AttVIB), and the other one to decrease it (AttCUT). Interestingly, their results show that the role of attention on the sensory stimuli has a major role in skill learning. They reported that when the practice followed AttVIB the performance was improved (along with less variability) when compared with AttCUT or no intervention. This was accompanied by changes in the cortical excitability (Rosenkranz & Rothwell 2012).

3. HOW IS A SKILL LEARN?

3.1 – The Concept of Skill and its Multistage Nature

Defining the concept of skill is hard, because it is a broad concept, common in multiple and disparate fields. Schmidt and Wrisberg (2008, pp. 4-9) defines it as a task or act with specific components and characteristics. A motor skill can be classified according to various criteria. Those different criteria, emphasise different aspect of the skill itself and the overall performance. The authors classified the skills according to the task organization, the relative importance to the motor and cognitive elements, and to the level of environmental predictability. The classification made by Schmidt and Wrisberg (2008, pp. 4-9) is summed up into table 1.

Since the concept of skill is broad, so it is the definition of motor skill learning (ML). It can be defined as a process of acquisition and subsequent refinement of a novel movement pattern (Kumpulainen et al. 2014), and an improvement of movement coordination (Latash 2010). ML can be studied according to different branches of movement science. It is however interesting to note that various components are included in the learning process. Schmidt and Wrisberg (2008, pp. 16-18) suggest that the at least 3 components participate to the learning process, that is the person, the type of skill itself, and the environment. The person is the most important component. In fact, multiple personal aspects (anatomical, developmental, psychological and affective) affect the learning process and the rate of learning. The second component is the type of skill itself. Table 1 suggests that there are different skills, with different requirements in terms of decision-making, adaptability, as well as the rhythmicity of the movement, and that a single skill can be categorized differently according to the characteristics of the skill that are emphasized. These requirements affect the way a skill is learned and the relative difficulty of the skill when coupled with the characteristics of the performer. Last, but certainly not least, the environment is important in the motor learning process. The stability of the environment affects not only the movement coordination, but affect the way the skill is learned. If the environment is unstable, the performer will be required to constantly adapt the movement to the environmental stimuli. On the other hand, if the environment is predictable, the performer does not have to adjust the performance according to the stimuli presented by the environment. A good example to show the difference is to analyse the differences in the powerlifting, compared with any sport in which the performer faces an opponent, like basketball, rugby, tennis, etc. Please note that even in stable environments, there is always a certain level of unpredictability, which represent a constant characteristics of nature.

TABLE 1 – According to the emphasis posed, a skill can be categorized in different ways. Adapted from (Schmidt & Wrisberg 2008, pp. 4-9)

Skills Classified by Task Organization	
Discrete Skills	Skills or tasks that are organized in such a way that the action is brief
Serial Skills	A type of skills characterized by a series of discrete actions connected together in a sequence
Continuous Skills	A type of skills in which the action in which the action unfolds without a recognizable beginning and end in a repetitive fashion
Skills Classified by the Relative Importance of Motor and Cognitive Elements	
Cognitive Skills	A skill for which the primary determinant of success is the quality of the performance itself, rather than the perceptual and decision making process.
Motor Skills	A skill for which the primary determinant of success is the quality of the performer’s decision regarding what to do.
Skills Classified by the Level of Environmental Predictability	
Open Skills	A skills performed in an environment that is unpredictable or in motion, and that requires the performer to adapt the performance to the dynamic properties of the environment.
Closed Skills	A skill performed in an environment that is predictable or stationary and that allows performance to plan the performance in advance.

Different branches of movement science study different aspects of the learning process. For example, ML can be studied from the neuroscientific perspective, to better understand the neural processes associated with the learning of a new skill. On the other hand, this process can be studied focusing on the behavioural changes, that is how the CNS controls and coordinates the body segments, and how the performance arises. Although those two points of view equally contribute to the understanding of motor learning, for the sake of this review only the neurophysiological aspects of motor learning will be exposed, as primarily related to the study. The author, however, strongly recommends the great reviews by Wu & Latash (2014) and Latash (2010) to expand the view of the learning process at a behavioural level. From whatever perspective the learning process is studied from, motor learning has traditionally been seen as a multi-stage process. The behavioural perspective has seen multiple model of motor learning. Although similar in principles, they differ on some subtle, but sometime remarkable features (Newel 1985; M. Latash 2010)

One of the first models of motor learning was provided by the Great Russian scientist Nikolai Bernstein, who proposed a 3 stage model. According to Bernstein, the first stage of ML was the freezing of the degrees of freedom (DOF), that was thought to be related to a simplification of the movement. This stage was followed by a progressive release of DOF reflecting a progressive mastering of the movement. The last stage of the model proposed by Bernstein was the exploration of the DOF. In this stage the movement was thought to be learned, and the subject can be considered a proficient performer (Latash 2010). The exploration of the DOF reflects a request of adaptation of the movement in various environmental conditions.

3.2 – Neurophysiology of Motor Skill Learning

3.2.1 – Central Mechanisms of Motor Skill Learning

Several studies have suggested that learning a task involves at least two stages. The first one, called fast learning, occurs within one single training session, whereas the second stage, called slow learning, sees further developments in the learning after multiple trainings. Between the stages, a process of consolidation ensures the strengthening of the learning (Dayan & Cohen 2011). Doyon & Benali (2005) suggested that learning a new skill actually includes 5 stages. In addition to the stages of fast and slow learning, the phases of consolidation, automation and the phase of retention have been proposed. However, these three further stages can be considered as sub-stages of a more generic skill consolidation stage.

Before discussing in more details the experimental data dealing with the role of the primary motor cortex (M1) in motor learning, it is useful to briefly discuss the more general mechanisms of motor learning, that is to expand the horizon to other brain structures (that are equally important for the learning adaptations). Various models have been proposed as central mechanisms of motor learning. One of them was proposed by Ungerleider & Doyon (2002). They proposed that the representational changes usually seen during the motor skill learning processes depends not only on the stage of learning, but also on the type of learning. They distinguished between learning a new sequence of movement and the adaptation of an existing skill to a change in environmental constraints.

Briefly, in the model proposed by Ungerleider & Doyon (2002), two systems (or loops) are proposed to be crucial for motor learning: the cortico-striato-thalamo-cortical (CS) and cortico-celebello-thalamo-cortical (CC) loop (figure 7). During the fast learning, the interaction between the celebral structures are the same for both type of learning. As the learning proceeds, changes in the representation can be observed. When the skill is well consolidated, the representation of the skill is distributed between the two circuits, with the CC system having an important role in motor adaptation, and the CS having a role in the motor sequence (Ungerleider & Doyon 2002).

Various authors have suggested that the primary motor cortex (M1) is an important site of motor learning. During the past years, with the help of the development of a great method of assessing M1, the Transcranial Magnetic Stimulation (TMS), multiple researches have shown that M1 is not just as the last site of the motor system, a mere representation of the muscles, but it is an active site of the learning adaptations. Structural changes in M1 occur, for example, according to the type of the sport (Kumpulainen et al. 2014), and stage of learning (Dayan & Cohen 2011). Different mechanisms are involved in these changes.

Kumpulainen et al. (2014) studied the difference in motor cortical excitability in athletes who performed mainly endurance vs. skill training. In order to compare the subjects, they used the paired associative stimulation (PAS). PAS intervention combines the stimulation of the nerve innervating the target muscle, followed by transcranial magnetic stimulation (TMS). According with the interval interstimulus (IS) and the order of stimulation, the PAS intervention can produce long-term potentiation (LTP)- or depression (LTD)-like plasticity (Ziemann et al. 2008; Carson & Kennedy 2013)

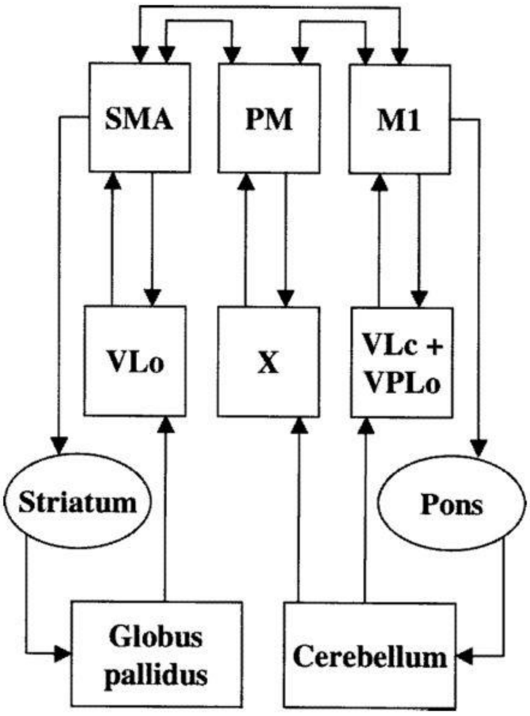


FIGURE 7 - The two systems involved in motor learning an adaptation proposed by (Ungerleider & Doyon 2002) and their interconnection. Cortical regions: SMA, Supplementary Motor Area; PM, Premotor Area; M1, Primary Motor Cortex. Thalamic Nuclei: VLo, Centrolateral Nucleus, oral division; X, Area X; VLc, Ventrolateral Nucleus, caudal division; VPLo, Ventroposterior Nucleus, oral division.

Using this intervention, they were able to show that in the soleus muscle there was a difference in the cortical plasticity between skilled and endurance athlete. Specifically, the PAS intervention produced an LTP-like plasticity in the skilled athlete, with no difference in pre- and post-PAS intervention in endurance athletes. They concluded that skill training might induce task-related adaptation in the motor cortex, thus enhancing the motor learning.

Different modulation in the motor cortex can occur during various phases of motor learning. Pascual-Leone et al. (1995) mapped the motor cortex expansion during a piano sequential movement routine. Using TMS mapping, they were able to show that during the initial stage of motor learning, there was an initial expansion of the cortical areas of the tested fingers, along with a decrease of the activation threshold (figure 8). This modulation of cortical output was suggested to be associated with an unmasking or strengthening of neural connection. A decade later, Rosenkranz et al. (2007) proved the original hypothesis of Pascual-Leone et al. (1995) to be right. They studied the difference of modulation in cortical plasticity in early and late phase of ML. Using again a PAS intervention and the MEP recruitment curve (discussed later in the text), they showed that the various phases of motor learning are associated with different cortical mechanisms. They suggested that the early phase of the ML is associated with an unmasking of neural connections, whereas the later phase of ML is suggested to occur via synaptogenesis.

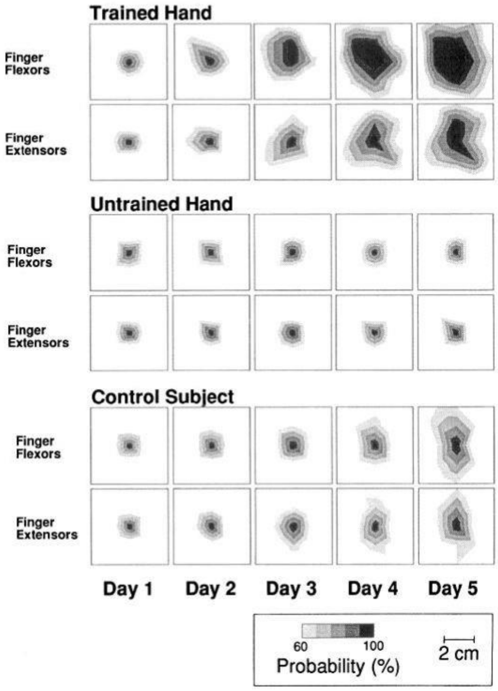


Figure 8 – In their experiment, Pascual-Leone et al. (1995) were able to show that the training elicits enlargement of the cortical representational ready over shot period of time.

In addition to PAS and the TMS mapping, a great tool to study the changes in corticospinal excitability after a training programme, is the input/output relationship of MEPs, also known as MEP recruitment curve. Its methodological consideration will be discussed later, in chapter 4. Experimental data suggest that the MEP recruitment curve is a great tool for longitudinal studies of the motor cortical excitability (Carroll et al. 2001). Using the MEP recruitment curve, several authors have shown that after a skill training, there is a shift of the MEP recruitment curve towards the left, which is interpreted as an increased in the corticospinal excitability (Rosenkranz et al. 2007; Perez et al. 2004; Tyč & Boyadjian 2011). In addition Tyč & Boyadjian (2011) linked this increase in the corticospinal excitability to a shift in the TMS map during multi-joint training (game of darts). They studied the changes in the TMS map size and its shift by recording the MEPs at the shoulder and the brachioradialis muscle. They were able to show that after six weeks of training the neural map of those two muscle (which are not anatomically close with each other) shifted towards each other, that is their activation site got closer. This was linked to a shift towards the left of the MEP recruitment curve of both muscles.

It is, therefore, clear that a skill acquisition process recruits large portion of the motor cortex during the first stage of learning. Once the skill is learned, however, the recruitment of M1 seems to decrease. This was suggested by Pascual-Leone et al. (1994), who showed that the cortical representation of the trained muscle decreased once the skill was learned, despite the training continued. Specifically, they reported that when the learning was in its implicit phase, the cortical engagement increased, but when the learning became explicit, the topography of the cortical map returned to the baseline. They suggested that the increased cortical representation are indicative of a learning process, rather than the performance itself (Nielsen & Cohen 2008).

3.2.2 – Peripheral Mechanisms of Motor Skill Learning

For long the role of the spinal cord was thought to be just a connection between the brain and the muscle (Wolpaw 2007). However, studies done in the past years, have suggested that the spinal cord has a functional role in movement production. This, not only because is the site of the involuntary movements, but also because of the recognition of the role of these involuntary mechanisms in movement production.

For example, Wolpaw et al. (1983), were interested in the adaptability of the stretch reflex. They trained monkeys to maintain a certain EMG activity in the biceps brachii while acting against an extensive torque. At random times, a sudden increase of the torque evoked a stretch reflex. The monkeys were given reward only if the EMG value after 14 to 24 ms after the stretch was above or below a set value. With this protocol, they showed a modulation of the stretch reflex accordingly with the condition of the EMG being under or above the set value. Wolpaw (2007) suggested that since the stretch reflex was evoked at an unpredictable time, and since a reflex is the earliest possible response that the CNS can use to react to an environmental perturbation, a change in the descending influence over the spinal cord must be the mechanism of adaptation. These descending signals have been shown to originate from the corticospinal tract, as suggested when the sensorimotor function was removed, or the corticospinal tract cut (Wolpaw 2007 for a review).

Another example comes from Nielsen et al. (1993), who studied the difference in H-reflex between professional dancers compared with untrained, moderately and well-trained subjects. Briefly, the H-reflex is the laboratory counterpart of the stretch reflex, and it has been extensively used in literature to measure the overall spinal excitability (Palmieri et al. 2004 for a review on the H-Reflex). They showed that the H_{max}/M_{max} ratio was significantly larger for the moderately and well-trained subjects compared with the untrained. However, the ratio was lower for the ballet dancer. They suggested that this result was due to an increase of pre-synaptical inhibition, possibly due to a large use of the co-contractions when keeping the ballet postures. They, therefore, concluded that the daily activities can have an effect on the spinal mechanisms of motor control, like the case of the co-contractions.

Taken all together, with other studies showing different functional changes in the spinal excitability, it is possible to conclude that even the spinal cord shows activity-dependent mechanisms. It has been suggested that these adaptation, just like the central structures, occur through LTP-like mechanisms (Wolpaw 2007; Inglis et al. 2000).

4. INVESTIGATING MOTOR SKILL LEARNING USING TRANSCRANIAL MAGNETIC STIMULATION

4.1 – Introduction to TMS

In the previous chapter, the neurophysiological aspects of motor learning have been discussed. Several methods have been used to assess the neurophysiology of motor learning. For example, several authors have used fMRI, which has been suggested to be the "golden standard" for the neuroimaging techniques (i.e. Karni et al. 1995). Other authors, however, have used transcranial magnetic stimulation (TMS) to directly stimulating the primary motor cortex (M1). The two methods are different in their nature since fMRI allows us to "see" what areas of the brain are activated by a specific action, and its changes over time (McIntyre et al. 2003). On the other hand, TMS allows us to directly stimulate (not only) the corticospinal neurons, which are included in the neuromuscular system (Rothwell 2011). As we shall see in this chapter, the results that TMS provides have helped to increase our understanding on the neuromechanics and the control of human movement.

TMS was originally developed by Barker et al. (1985), and it can be considered as the "son" of the transcranial electric stimulation (TES) developed by Merton & Morton (1980). Its functioning is based on magnetic fields. If a brief and high voltage current is passed through the TMS coil, a magnetic field is produced. This magnetic field passes through the scalp and to the cortex virtually unimpeded and in a painlessly way. If the magnetic field is strong enough, the neuron is excited and fires (Terao & Ugawa 2002). The reader who wishes to gain more information about the physics and the functioning of the TMS, which are beyond the purpose of this chapter, the book by Rotenberg et al. (2014) is highly recommended.

Due to its physical properties, the TMS can rarely reach subcortical structures, since the magnetic field strength decay as function of the depth of the travelling pulse (Epstein et al. 1990). This makes TMS a safe way to stimulate the brain. Before moving on to the explanation of the various TMS physiology and the various paradigms, it is useful to have a brief exposition of the cortex anatomy, since multiple type of neuron can be stimulated by the TMS magnetic field.

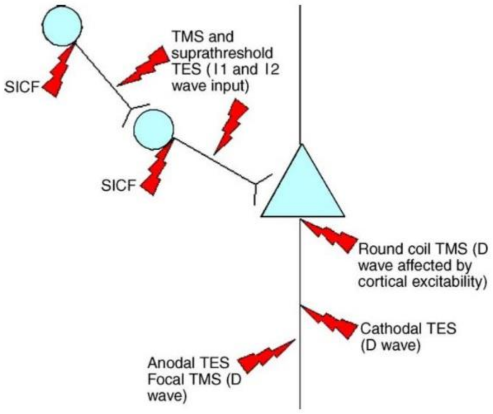
4.1.1 – A Brief Anatomy of the Cerebral Cortex and the Physiology of the TMS

The cerebral cortex is the evolutionary younger part of the brain, and it is formed by four lobes. Its surface is highly convoluted and, for this reason, on its surface it is possible to note sulci (grooves) and gyri (elevated regions) (Kandel et al. 2013, pp. 5-15).

The cerebral cortex has two major classes of neurons. The first type is called projection neurons, while the second class are the interneurons. The projection neurons have their cell body within one layer of the cortex, but their axon leave the layer to convey information between layers. An example of projector neurons are the pyramidal neurons that, from the cortex, convey the descending signals to the subcortical levels of the motor system. The second class of neurons inside the cortex are the interneurons. These neurons have their axons within the layer, therefore making part of a “local network”. According to the neurotransmitter that those interneurons use, it is possible to distinguish between inhibitory and excitatory interneurons (Kandel et al. 2013, pp. 210-235). The inhibitory interneurons use γ-amminobutyric acid (GABA) as neurotramitter, while the excitatory interneurons use glutamate to convey information (Wonders & Anderson 2006)

The cortex is organized in layers and columns. The columns run throughout the thickness of the cortex and are considered the fundamental computational modules of the cortex (DeFelipe et al. 2012). This because the neurons within the same column tend to have the same responsive properties (Kandel et al. 2013, pp. 237-255). In addition to the columns, the cortex is organized in 6 layers. The thickness of each layer is based on the specific cortical area. The reader who would like to gain more information about the layers, appendix 1 at the end of the thesis contains a brief presentation of the neurons contained in each layer and the function of each layer.

In the introduction, it has been suggested that TMS can be considered as the evolution of TES. However, there are some major difference that have to be considered, when comparing the methods and, more importantly, the results. The main important difference between TMS and TES is the site of stimulation. In fact, studies have shown that TMS is likely to activate the corticospinal neuron indirectly, that is pre-synaptically or at the neurons soma, while TES is likely to activate this neuron directly at the axon level (figure 9). In practice, this translates in MEPs produced by TES that are around 1.5 ms faster that the MEPs evoked by TMS. At the spinal level, this early response of TES has been called D-wave (direct). On the other hand, TMS produces multiple I-waves (indirect). These I-waves are enumerated based on their latency (Di Lazzaro et al. 2004).



FIGEURE 9 - A TMS pulse can act at different site of the corticospinal tract. In this schematic view a projection neuron (triangular shape) and two interneurons (round shape) are represented. Image from Di Lazzaro et al. (2004)

4.1.2 - What Does TMS measures?

It has to be noted, that when we stimulate the brain using TMS, we do not exactly know what type and how many neurons we are stimulating. This means that the MEP, in studies on human movement, almost surely reflects a mix of excitatory and inhibitory mechanisms from various type of neurons. What we have as a result of a TMS pulse is the NET effect of stimulating a relatively large area (in comparison with the size of a neuron) of the brain (Di Lazzaro et al. 2004). In addition, as suggested by Di Lazzaro et al. (2004), the relationship between the TMS pulse and the EMG response in the muscle is not straight forward. This because it is assumed that the descending volleys from a certain TMS pulse are directed all to the target muscle, but this might not be the case, and some neurons that are stimulated with the pulse might project to other muscles rather than the target muscle. Lastly, different TMS coils have different degrees of focality (Cohen et al. 1990), which reflect different sizes of brain area that are stimulated.

Another aspect of TMS that is interesting to point out is that the MEP recorded in the EMG trace provides information on the excitability of a certain neural population without, however, providing information on the information itself. Although this might seem a minor point, it is important for the purpose of the motor control that, as stated earlier in the text, is concerned with modelling the data that neurophysiology and behavioural studies provide into a model of how the CNS control and coordinates the body in the environment.

Keeping in mind these drawbacks, there is no doubt that TMS provides fundamental information about the physiology of the corticospinal system that no other methods are able to provide. Depending on the type of stimulation paradigm used, several parameters have been measured, each of which providing information to several aspects of the motor system physiology. For reason of brevity, in this manuscript only the techniques and the parameters relevant to present study will be exposed. Those parameters are the input/output curve and the short-interval intracortical inhibition (SICI).

4.2 – The Input/Output Curve

Also known as MEP recruitment curve, this parameter provides useful information about the corticospinal excitability at different stimulus intensities. When the MEP response is plotted against the stimulation intensity, the result is a sigmoid curve (figure 10). At a certain stimulation intensity, this curve reaches the plateau, that is no matter the increase in the stimulation intensity, the response will not increase (Devanne et al. 1997).

Devanne et al. (1997) were among the first to introduce the IO relation of the MEP as neurophysiological parameter. They showed for the first time, that the relation between stimulus intensity and MEP is not linear, but has a sigmoidal shape. They suggested that this feature of the IO relation might be due to various reasons, including the way the neurons are recruited by the magnetic pulse, the multiple components of the corticospinal tract, as well as the fact that progressively larger motor units are recruited with the increase of the stimulus (Devanne et al. 1997). In addition, they suggested that three parameters characterize the IO relation, that is the threshold of rise of the curve, the slope of the sigmoidal curve, and the plateau of the curve. The latter, was suggested to represent a balance between the cortical excitatory and inhibitory mechanisms, rather than the maximum firing capacity of the neuronal population (Devanne et al. 1997).

Since then, the IO curve has been used in several occasions as an indicator of corticospinal excitability, and has been applied to a plethora of research questions. One interesting area in which the IO relation has been applied is motor learning. Carroll et al. (2001) were interested in the repeatability of this parameter during multiple tests, an important requirement for studies of motor learning. One of their main concerns, in fact, is that TMS measures can be altered by various parameters that are not easy to control. For example, if the coil is slightly moved, different neurons may be stimulated. Although this can be controlled up to a certain degree during a single TMS session, it is not possible to control when the experimental design requires multiple measurements. Their results showed that the IO curve can be used as a reliable index of corticospinal excitability in longitudinal studies. In addition, they suggested that the parameters aforementioned, defined by Devanne et al. (1997), could be taken as parameters to describe changes in the neurophysiology over time. However, Carroll et al. (2001) suggested that the best parameters to describe changes in the IO curve during longitudinal studies might be represented by the x-intercept of the peak slope, which they suggested to be less susceptible to experimental errors.

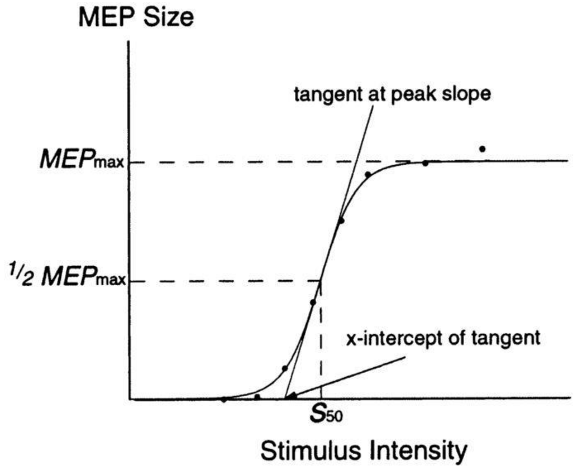


FIGURE 10 -If the MEP is plotted against the stimulus intensity, the resulting line is a sigmoid curve, with a threshold, a slope and a plateau. From (Devanne et al. 1997)

Several studies aiming at the assessment of the changes in the corticospinal system with motor learning have used the slope of the IO curve as an index of motor learning. If the muscle activation is kept constant, say in resting condition, then a bigger MEP at the same stimulation intensity (SI) shows that the neurons have become more excitable. Because the IO curve assesses various SI, then the resultant slope of the curve shifts according to the excitability of the neurons (Devanne et al. 1997). A shift toward the left of the slope of the IO curve has been associated with motor learning, since the neurons become more excitable (Rosenkranz et al. 2007).

4.3 – The Short Intracortical Inhibition (SICI)

TMS is a good tool to assess the corticospinal excitability. As exposed earlier, this is done using single pulse paradigms. However, TMS can be a great tool to assess other circuits rather than the corticospinal tract. If a couple of pulses are given at a certain interstimulus interval (ISI), then TMS can be useful to assess the cortico-cortical and transcallosal networks (Kobayashi & Pascual-Leone 2003). These paradigms are called paired-pulse. In these paradigms, a first stimulus (S1 or conditioning stimulus, CS) has the role of conditioning the second stimulus (S2 or test stimulus, TS). According to the intensity of the CS and the ISI, that is the timing between the 2 stimuli, the conditioned MEP can increase or decrease, when compared with unconditioned MEP. The researcher can obtain inhibition of facilitation of the corticospinal excitability by manipulating the stimulus intensity, as well as the ISI (figure 11).

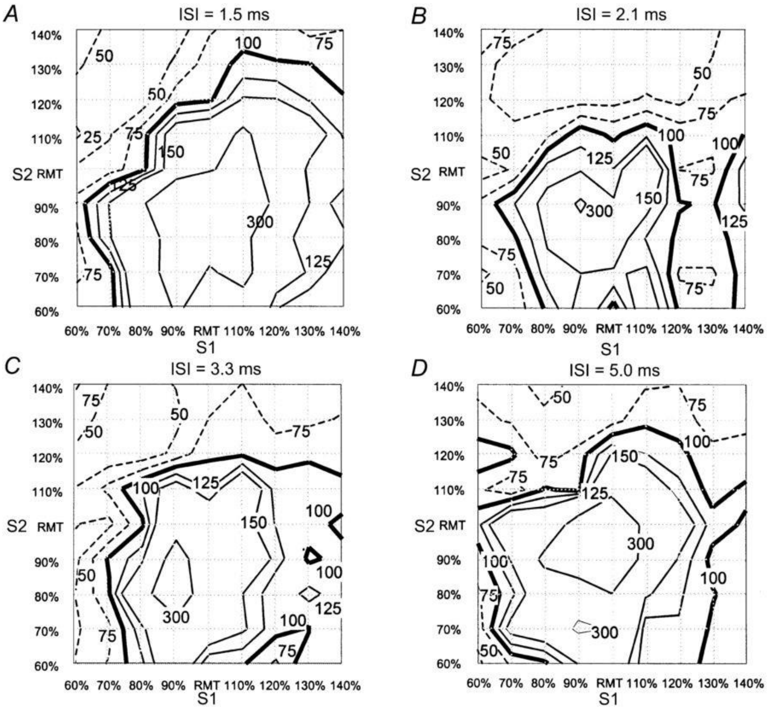


FIGURE 11 - According to the intensity of CS and TS, as well as the timing of ISI, Inhibition or facilitation of the corticospinal excitability can be obtained. Results from Ilić et al. (2002). The thick line represents no interaction of the two stimuli, the dashed line show inhibition, while the continuous line represent facilitation. SI are expressed as percentage of rMT.

At the beginning of the 90’s, Kujirai et al. (1993) showed for the first time that if the CS was lower than the motor threshold and the TS higher, with the ISI ranged from 1 to 5 ms, the conditioned MEP was lower than the unconditioned. In other word, the conditioning MEP inhibited the TS. This mechanism is thought to involve the low-threshold, GABA-ergic inhibitory interneurons. They are called low threshold because they are activated with a lower induced-current compared with the corticospinal neurons. This was proven by Di Lazzaro et al. (1998). They used the paired-pulse paradigm in patients with spinal cord stimulator, so that they could monitor all the I-waves produced by the TMS pulse. They showed that the I1 was unaffected by the CS, while it produced an inhibition in the later waves. This was interpreted as an interaction of other neurons, rather than a direct inhibition of the corticospinal neuron itself.

Some functional meaning of SICI has been already discussed in chapter 3. The mechanism of SICI has been suggested by Floeter & Rothwell (1999) to be similar as "*releasing the brakes before pressing the gas pedal*". In fact, it has been reported that the inhibition of the neurons projecting to the muscle to be contracted is lower compared with its antagonist (which remain relaxed). It was suggested, therefore that the excitability of the corticospinal neurons is under the influence of the inhibitory interneurons, which indirectly control the movement production. This also explain the results of Rosenkranz & Rothwell (2003) exposed earlier in the text, when talking about sensorimotor integration.

5. THE PURPOSE OF THE STUDY

The purpose of the present study is to assess the possibility that a motor skill training based on transient visual deprivation can produce a better motor learning, from both the neurophysiological and the performance point of view. To the knowledge of the author, no previous researchers have used a blindfold in motor learning studies. However, this concept has been used, and it is still used in practical setups, with the belief that this might improve the technique and the general motor learning.

Although no direct studies have directly assessed this possibility, it seems plausible to think, given the literature discussed earlier in the text, that this concept could be promising. It has been shown by Leon-Sarmiento et al. (2005) that 30 minutes of continuous light deprivation, either with eyes opened and closed is associated with an increased cortical excitability when compared with normal light conditions. From this study, the question raises whether this increase in the corticospinal excitability can be used to enhance the learning process. Although in the previous sections it has been shown that vision might have the dominance in the control of the movement (Sarlegna & Sainburg 2009), the study by Mon-Williams et al. (1997) suggests that when vision is not available, the proprioception seems to be dominant. Another important concept associated with the this concept is the fact that it has been sown that attention to the proprioceptive feedback is associated with a modulation, in the direction of the increase of the corticospinal excitability (Rosenkranz & Rothwell 2012).

Thus, it is possible to hypothesise that if the performer poses the attention on the proprioceptive signals coming in the form of awareness of where the body is in the space, this could improve the sensorimotor integration, which in turn would results in an increase in the corticospinal excitability, an improve in coordination and the overall performance. The present study tests this hypothesis.

6. METHODS

6.1 – Participants and Study Groups

10 Right-Handed, physically active university students volunteered to participate to the study. The subjects were recruited according to three inclusion criteria: 1) right-handedness; 2) physical activity level; and 3) sport history. The latter was chosen as a precaution, as it has been reported that coordinative, multi-joint movements can change the neural map of the motor cortex (Tyč & Boyadjian 2011). Thus, it was required from the subjects not to have a history of professional (or competitive) training in sports in which the fine control of the upper limb was the main part of the sport technique. Examples of sports which precluded the participation of the study were tennis, volleyball, basketball.

The subjects were randomly divided in two groups. The BLIND group performed the measurements and the motor practice blindfolded. The subjects in this group were allowed to use their vision only for a restricted period, while receiving the feedback after each trial. The control group, named NORM, performed the measurements and the motor practice without any visual restriction. Both groups performed the same measurements, with the only difference being the visual allowance. The BLIND group performed an additional measurement in order to assess if the blindfolding itself produced significant changes in the corticospinal excitability while resting. These additional measurements were called “BLIND measurements” (see later for more details).

All the subjects were volunteers, and no compensation was provided for the participation to the study. Prior to the participation, the participants were informed about the protocols, and the risk associated with the participation. In addition, In order to screen them against possible contraindication of TMS, the participants were asked to fill a TMS screening questionnaire (Rossi et al. 2011). The reader will find the informed consent and the TMS screening questionnaire in the appendix 2. All the methods have been approved by the local university ethic committee, in accordance with the declaration of Helsinki.

6.2 – Experimental Design

Both groups took part in a skill training program that lasted five days (table 2). The neurophysiological measurements included PRE and POST measurements. The PRE measurements were performed on either Monday or Tuesday, depending on the availability of the subject, the laboratory and the measurements schedule. The POST measurements were then performed on Friday or Saturday, respectively. On day 1 (henceforth D1) and day 5 (henceforth D5), the subjects performed the same procedure. In the PRE and POST measurement in both D1 and D5, the input/output relationship (IO curve) of the motor-evoked potentials (MEPs) and the short-interval intracortical inhibition (SICI) curves were assessed. In between PRE and POST measurements the first and the last training, respectively, were performed. During D2, D3 and D4, the subjects performed only the motor practice that lasted around 30 minutes per day. Table 2 shows a summary of the protocol design. We chose to assess the CSE on both D1 and D5 because it has been reported that the learning process can be detected even after one single training session, a phenomenon that has been named “fast learning” (Dayan & Cohen 2011) . See chapter 3 for more details on motor learning.

TABLE 2 – A schematic representation of the experimental design. training programme consisted in 5 consecutive days of motor practice. At the beginning and the end of the programme, neurophysiological measure of corticospinal excitability was collected. The BLIND group underwent to an additional measure (in the table “Day 0”), which was done at least the week before the beginning of the training program.

Day 0	Day 1	Day 2-4	Day 5
BLIND Measurements (For BLIND Group only)	IO curve PRE D1 SICI PRE D1	Practice (30’)	IO curve PRE D5 SICI PRE D5
	1st Practice (30’)		Last Practice (30’)
	IO curve POST D1 SICI POST D1		IO curve POST D5 SICI POST D5

6.3 – Neurophysiological Measurements

Electromyography. The MEPs were recorded at the right bicep brachii (BB) using self-adhesive, mono-use surface EMG electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm^2). The electrodes were arranged in a pseudo-unipolar arrangement (Hoffman et al. 2009). This arrangement was preferred to the bipolar configuration because during the pilot study we noticed that the pseudo-unipolar arrangement allowed us to evoke MEPs of higher amplitude and better quality. In particular, the “pseudo-unipolar” arrangement allowed for MEPs of standardized shape, making easier the analysis of their amplitude. This type of unipolar electrodes placement, depicted in figure 12, consisted of an electrode on the biceps, and the other on the back of the shoulder, in proximity of the scapular bone. The reference electrode was placed on the external side of the right wrist, on the bone. The electrode on the BB was placed according the SENIAM recommendation (Surface Electromyography for Non-Invasive Assessment of Muscles). For the BB, these recommendations suggested that the electrode should be place at 1/3 on the length of the BB from the fossa cubit. Once the correct placement of the electrodes was found, the skin area was shaved, abrased and cleaned using alcohol. This was done in order to decrease the resistance. The skin resistance was then checked using a multimeter (Fluke 87V, Fluke, USA). Specifically, we made sure that the electrode resistance was below 5 kΩ. In this study, this value never went above 3 kΩ.

The raw EMG signal was amplified (100x) and high-pass filtered (10 Hz cut-off frequency) by a preamplifier (NL824, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK), and to this it followed a band-pass filter (10-1000 Hz) by a custom-made amplifier (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland). The signal was sampled and recorded at 2000 Hz using an A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK) and Spike2 software (version 6.10, Cambridge Electronics Design, Cambridge, UK). Appendix 3 shows a samples of the MEPs for all the measurement parameters.

Transcranial Magnetic Stimulation (TMS) Procedure. Prior to any TMS measurements, the hotspot for the BB was found and marked on the scalp of the subjects. The procedure begun by placing the coil at the distance of 2 cm backward and 5 cm leftward to the vertex. This placement was chosen because during the pilot measurements we observed that this starting point was around BB hotspot for TMS measurements. From there, the hotspot was defined as the coil position in which the biggest MEP amplitude was obtained, at the same stimulus intensity. Once the hotspot was found, the external edges of the TMS coil was marked on the skin, and the coil placed on a custom-made coil holder. Efforts were made not to move the coil from the defined position. The passive and active motor thresholds were then measured. The resting motor threshold (rMT) was defined as the minimum stimulus intensity in which a clear MEP was visible in 3 out of 5 trials for that given intensity. The same procedure was repeated for the active motor threshold (aMT), which was defined as the minimum stimulus intensity in which a clear MEP was visible in 3 out of 5 trials for that given intensity, while the subject performed an isometric arm flexion, with 10% of the MVC. The MVC was found before the electrode placement, so that the subject had time to recover, and was defined as the maximum weight that the subject could lift in an arm flexion.

The TMS stimulation pulses were delivered on the contralateral motor cortex using a Magstim Bistim[2] (Magstim, Whitland, UK). A 7 cm flat, figure-of-eight shaped TMS coil (Magstim, Whitland, UK) was held by the researcher, and was placed with the handle pointing backward and at around 45 degrees leftward, as previously found by Ahmed Abdalla (2011). During the TMS measurements, the subjects were asked to perform an attentional task, consisting in a countdown from 200 to 0 (Kumpulainen et al. 2014). In addition, they were instructed to move as little as possible, and "to think of nothing, especially their body". Each block of the TMS measurement, (IO curve, SICI Curve, etc.) never lasted more than 15 minutes, and the subject was released and allowed to freely walk in between the blocks for at least 5 minutes. This was important to keep the subject awake and motivate them to stay still during the measurements. In addition, it was important for the BLIND group to avoid modulations of the CSE with prolonged visual deprivation. (Leon-Sarmiento et al. 2005)

FIGURE 12 – The Electrode Placement. An electrode was placed at the biceps brachii level, while the other was placed in proximity of the scapular bone. Lastly, a reference electrode was placed on the wrist bone.

Input-Output (IO) Curve. The IO curve was obtained by plotting the MEP response against various stimulus intensities (Carroll et al. 2001). The stimulation intensities of 90%, 100% (rMT), 110% 120%, 130% and 140% of the rMT were chosen. For each intensity, 10 stimulations were given and then saved on the laboratory computer for offline analysis. The various intensity blocks changed randomly, with a short break (max 2 minutes) in between the intensities to give time for the researcher to save the files and for the subjects to relax. The interstimulus interval was set between 7 and 10 seconds.

Short-Interval Intracortical Inhibition (SICI). SICI is a paired-pulse paradigm in which a conditioning stimulus (CS) is given before a test stimulus (TS), at a certain interstimulus interval (ISI). If the CS intensity is lower than the TS, and the ISI is between 1 and 5 ms, the peak-to-peak amplitude of the latter (TS) MEP is reduced. (Ilić et al. 2002; Rossini et al. 2015). In this study, SICI was tested using 3 CS intensities. The CS was set at 70%, 80% and 90% of the aMT, while the test stimulus (TS) was set at 130% of the rMT. The ISI was set at 2 ms. For each CS intensity, 8 unconditioned MEPs (that is the TS alone), and 8 conditioned MEPs were recorded. The unconditioned MEPS were recorded for each CS intensity.

BLIND Measurements. Leon-Sarmiento et al. (2005) reported that in resting conditions, after 30 minutes of blindfolding either with eyes opened or closed, the motor cortical excitability increased, compared with the light condition. It was therefore important for the assessment of possible benefit of a skill training performed blindfolded to determine if this modulation previously described was present for shorter period of visual deprivation. In the present study, the BLIND group performed the measurements and the training program blindfolded, however, the subjects were allowed to remove the blindfold to receive the feedback, which lasted less than 15 seconds.

In order to test the hypothesis that the blindfolding itself could produce any change in motor cortical excitability when the subjects repeated a practice-like blindfolding pattern, without any movement, as well as determining the short-term effects of blindfolding, the BLIND group performed an additional measurement, that was called "BLIND measurements". In order to test that, MEPs and SICI were recorded at specific timings. MEPs were measured using the intensity set at 130% of rMT, while SICI was recorded with the CS set at 80% of aMT, and the TS set at 130% of rMT, with and ISI of 2 ms. The number of stimulation were 8 for both MEPs and SICI stimulations.

In chronological order the MEPs and SICI PRE were measured first in light conditions. After that, MEPs and SICI were measured 15 seconds after the blindfold application to the resting subjects (P15”B), and 15 seconds after the blindfold removal (P15”N). In both cases the blindfold application was controlled by the researcher These tests were used to have insights in the short-term effects of the blindfolding on the CSE. In addition to those parameters, measures of MEPs and SICI after 10 (P10), 20 (P20) and 30 (P30) minutes after the subject reproduced the pattern of blindfolding was recorded. The MEPs and the SICI measured after 30 minutes, were considered as POST measurements. The blindfolding pattern consisted of 15 seconds of blindfolding and 15 seconds of non-blindfolding. The timing was dictated by a timer, which produced a "beep" every 15 seconds. The blindfold was held in place by the subjects themselves, with the left hand. This pattern was chosen because the target line lasted around 15”, and the feedback during the practice did not last more than 15 seconds. MEPs and SICI measured P15”B, P15”N, P10, P20, P30 were recorded with the blindfold on first and then the blindfold was removed for the non-blindfolded measures. The stimulations begun after 15 seconds in both cases.

6.4 – Motor Practice

The motor practice was done using a custom-made device, depicted in figure 13 (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland). The device included a handle connected to a weight via a pulley. Connected to the pulley, there was a potentiometer that transformed the movement of the pulley, in cursor trajectory, on the computer screen. The subjects sat comfortably on a custom-made chair (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland). The distance between the device and the chair was regulated for each subject, in order to match their comfort, and to match the subject arm's range of movement, with the cable excursion. This distance was kept constant throughout the whole practice period.

The performance task consisted of a visuomotor trajectory (figure 14), that was previously created by the researchers. This movement trajectory was the same for both groups, and was composed of 3 major “components”, that is big movements, small movements and incremental/decremental movements. The performance task lasted around 15 seconds.

FIGURE 13 - The set up consisted of a custom-made pulley. The length of the chair was adjusted to allow the full range of motion.

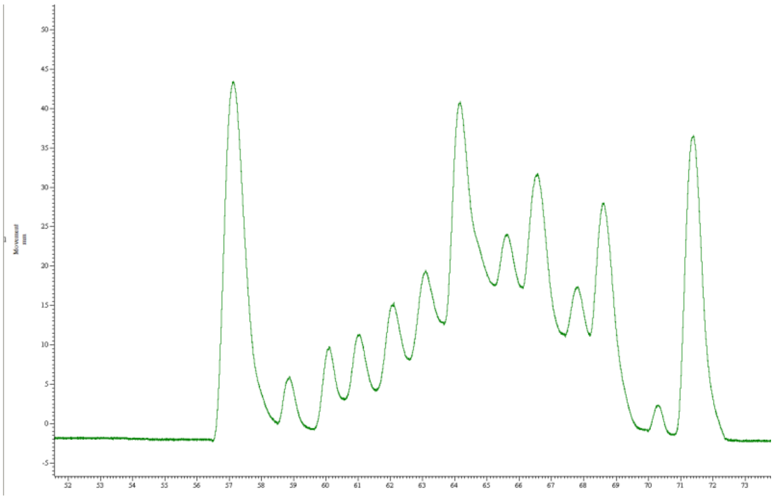


Figure 14. The Performance task consisted of a mix of big movements, small movements, incremental and decremental components.

At the "go" of the researchers, the subjects begun the movements. Since both groups were not allowed to have online feedback, in case the movement produced exceeded the performance task timing, a "stop" by the researchers ratified the end of the trial. At the end of each trial, the subject received the feedback. A different window showed the performance task on the background, and the front of it the trajectory the subjects performed. The feedback was visually, and the researchers always encouraged the subjects to do better.

6.5 – Data Analysis and Statistics

Neurophysiological Parameters Analysis. All the intensities of the I/O recruitment curve and the SICI were saved as individual file. Each file was analysed using the software Spike 2 (version 6.10, Cambridge Design Electronics). Using a custom-made script, the peak-to-peak amplitude of the MEPs for each intensity was calculated. The intensities, were then exported on a Microsoft Office Excel 2016 (Microsoft, USA) file and, for each intensity, the responses were averaged. Because we did not record the M-max value, direct comparison of the I/O Curve between D1 and D5 was not possible. Therefore, the neurophysiological Parameters were expressed in the form of difference between PRE and POST conditions (post – pre values) for both D1 and D5. In addition, the overall corticospinal excitability (CSE) was calculated by averaging the MEPs of the entire I/O Curve (90% to 140%; Rosenkranz et al. 2007). The same was done for the SICI parameter. For the "BLIND measurements" it was not possible to record at each block both conditioned and unconditioned MEPs for the SICI analysis. Therefore, the conditioned MEPs were compared with the MEPs recorded before as measure of corticospinal excitability.

Performance analysis. The software Spike 2 was used to record the trainings, and each training was saved in a single file. This file was then exported as a MATLAB (version R2015a, The MathWorks, Inc, USA) Each trial was analysed using a custom-made MATLAB script. The file was first split in each trial, and then each trial was compared with the target line. The difference line was then averaged and taken as output measure for the trial. The average of all the trials was taken as performance level for the training day.

Statistical Analysis. All the results from the analysis were analysed for significance using SPSS (IBM SPSS Statistics, version 22, Chicago, IL, USA). First the normality of the distribution was checked using the Shapiro-Wilk test. This was chosen over the Kolmogorov-Smirnov test as the sample size was small. The difference between the groups for the IO and SICI curves and the curves averages were assessed using the independent sample t-test. The difference within the groups, that is the difference in the values between Day1 and Day 5 was assessed using the paired-sample t-test. The same procedure was done for the behavioural data.

7. RESULTS

7.1 – BLIND Measurements

The results reported below failed to report significance. Nonetheless, the blindfolding pattern used in the present study seems to produce a positive effect on the corticospinal excitability. The results show that 15” of blindfolding produced a small facilitatory effect on the CSE, in the form of increase in the MEPs value compared with PRE values (difference from PRE values: 0.09 ± 0.39). This effect was still in place after 15 second from the removal of the blindfold (difference from PRE values: 0.06 ± 0.36). Figure 15 shows the dynamics of the CSE during the 30 minutes of the tests. After 10 minutes of blindfolding pattern, there was a marked increase in the MEPs amplitude, both when the measurements were done blindfolded (difference from PRE values: 0.64 ± 0.74) or after the blindfold removal (difference from PRE values: 0.26 ± 0.95). This increase in the neurophysiological values were smaller, but still evident during the P20 (difference from PRE values blindfolded: 0.40 ± 0.95; normal: 0.29 ± 0.64) and P30 measurements. In particular, if the P30 measurements are compared with the PRE measurements, we can conclude that 30 minutes of blindfolding without any practice produces an increase in CSE, both when the measurements are done blindfolded (difference from PRE values: 0.34 ± 0.75) as well as after the blindfold removal (difference from PRE values: 0.20 ± 0.84).

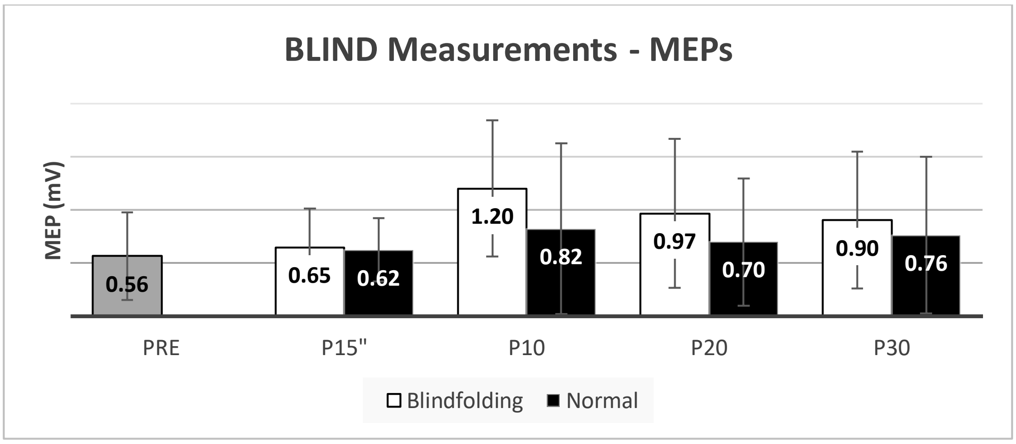


FIGURE 15 – The dynamics of the corticospinal excitability during the BLIND measurements. The group “Blindfolding” refers to the measurements performed while the subjects were

blindfolded (15” after the blindfold application), while the values under the group “Normal” refer to the measurements performed 15” of blindfold application. The “Blindfolding” measurements were recorded before the “normal”, and in both condition the subjects were resting.

The dynamics of SICI, depicted in figure 16, shows that after 15 seconds of blindfolding, the inhibition decreased compared from the PRE values, as the ratio between conditioned and unconditioned MEPs increased (difference from PRE values: 0.09 ± 0.53 mV). On the contrary, after 15 second of blindfold removal, the cortical inhibition slightly increased, as measure of a lower ratio (difference from PRE values: -0.04 ± 0.42 mV). The P10 and P20 showed a marked increase in inhibition when the subjects were blindfolded (difference from PRE values P10: -0.29 ± 0.35 mV; P20: -0.33 ± 0.42 mV), compared to the measurement after the blindfold removal (difference from PRE values P10: -0.17 ± 0.40 mV; P20: -0.12 ± 0.35 mV). P30 showed as well a slight increase in inhibition in the last 10 minutes of blindfolding pattern (difference from PRE values: -0.06 ± 0.44 mV). On the other hand, when the measurements were done non-blindfolded, the inhibition slightly decreased, and almost matched the PRE value (difference from PRE values: 0.03 ± 0.29 mV).

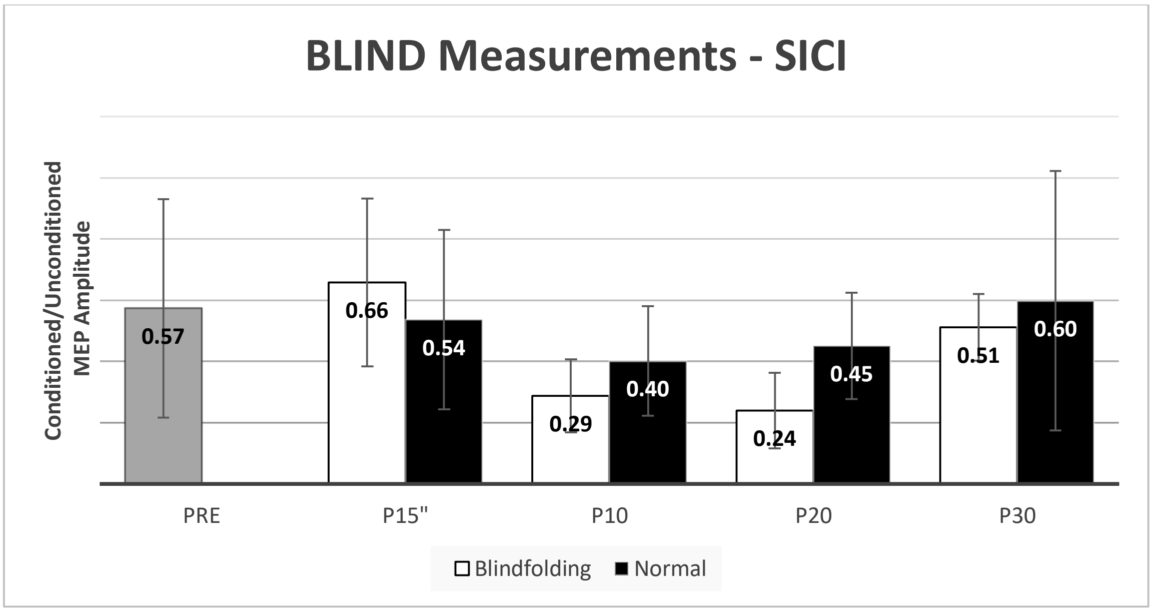


FIGURE 16 – The dynamics of the cortical inhibition during the BLIND measurements. The group “Blindfolding” refers to the measurements performed while the subjects were blindfolded (15” after the blindfold application), while the values under the group “Normal” refer to the measurements performed 15” after the blindfold removal.

7.2 – Neurophysiological Parameters of Motor Learning

Resting and Active Motor Threshold. The Training program did not produce any appreciable change in the resting (D1: BLIND: 50 ± 8, NORM: 49 ± 8; D5: BLIND: 52 ± 11, NORM: 48 ± 7) and active (D1: BLIND: 36 ± 4, NORM: 35 ± 7; D5: BLIND: 37 ± 4, NORM: 35 ± 5) motor threshold. The values presented represent the intensities of the TMS stimulator.

7.2.1 – I/O Recruitment Curve

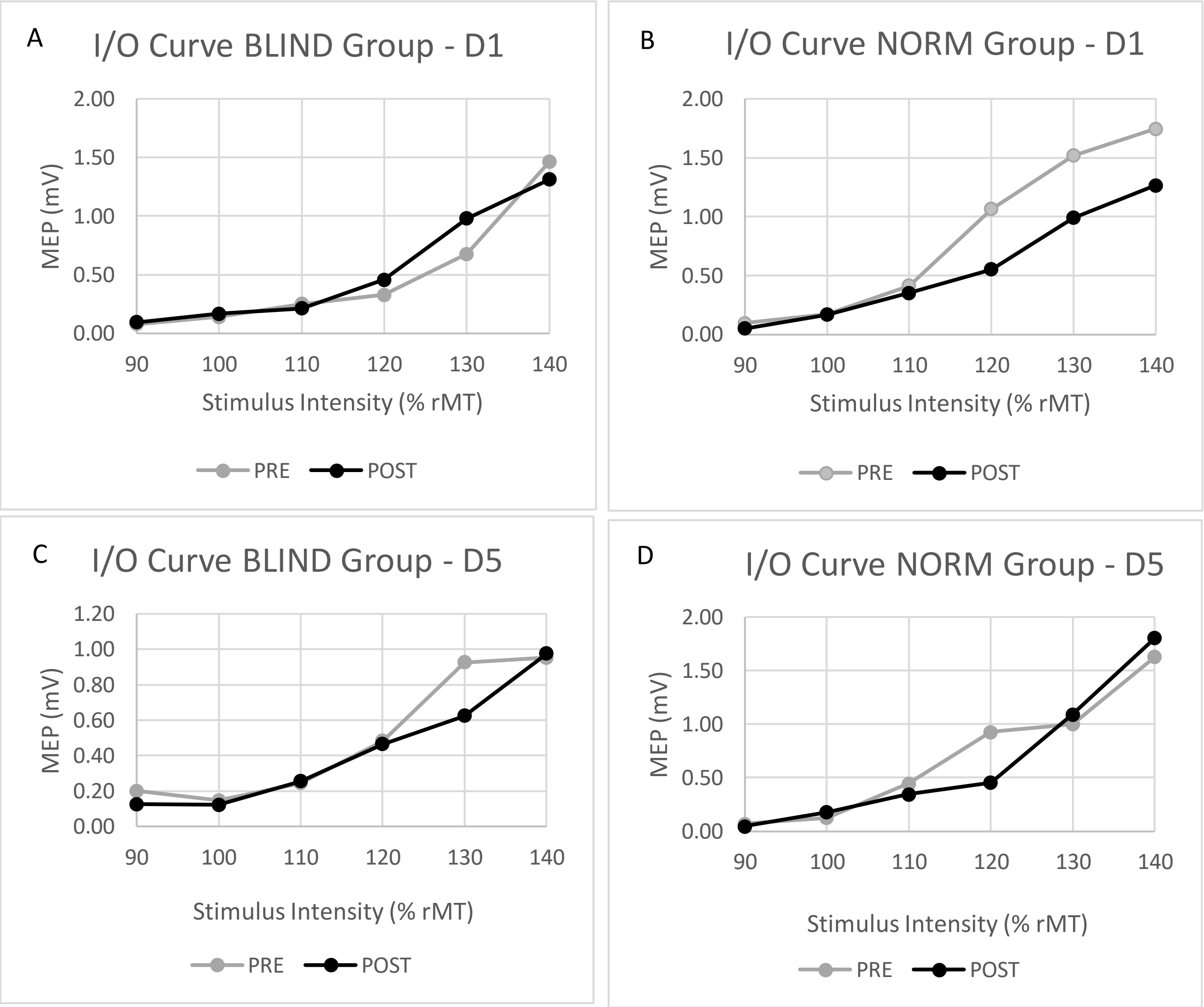


FIGURE 17 – The absolute values of the I/O curves of the two groups. A and C depict the I/O curves of the BLIND group in D1 and D5, respectively; B and D represent the I/O curves of the NORM group on D1 and D5, respectively. The PRE and POST curves represents the measurements performed prior and after the practice, respectively.

Both I/O recruitment curve and SICI are expressed not as absolute values, but we manipulated the values in order to compare the PRE and POST conditions, in both D1 and D5, by expressing the values as difference (post-pre) in the values. The statistical analysis of those indices did not show significant differences for most of the values.

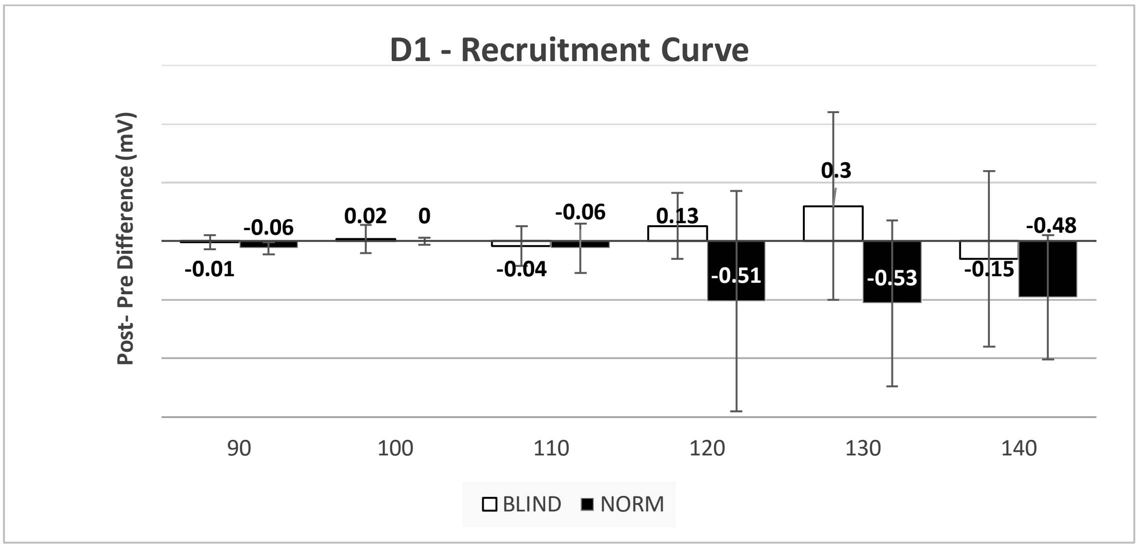


FIGURE 18 – The figure shows the difference between the post and the pre values for each intensity of the IO curve on day 1. The positive values represent an increase of the CSE in the post measurement. Conversely, when the value is negative, the CSE decreased in the post measurements.

Nonetheless, for exhibition purposes, figure 17 and 21 depict the absolute values of the I/O and the SICI curves.

Day 1. After the first training, The BLIND group showed a small increase in CSE, as figure 18 shows. This was suggested by an increase in the difference between post and pre training values, and it was evident in the latter portion of the I/O curve (120%: 0.13 ± 0.28 mV, 130%:0.30 ± 0.80 mV). On the contrary, the NORM group showed a reduction of the CSE. This was appreciable throughout the whole curve, but the difference between pre and post training was evident at higher stimulation intensities (120%: -0.51 ± 0.94 mV, 130%: -0.53 ± 0.71 mV; 140%: -0.48 ± 0.53 mV). Additional information was provided by averaging the MEPs of both I/O and SICI curves. This parameter provides insight to the overall excitability of the corticospinal tract, as suggested by other researchers (Rosenkranz et al. 2007). The dynamics of the pre-post training values (showed in figure 20), suggested that the training had different effects on the CSE. Indeed, for the BLIND group, the first practice induced and increase in the overall excitability of the corticospinal tract (0.07 ± 0.16). On the other hand, for the NORM group, the practice produced a substantial decrease in the overall excitability (-0.22 ± 0.26 mV).

Day 5. On D5, no substantial changes in the dynamics of the CSE during the last training for the BLIND group was observed, as figure 19 shows. However, at higher intensities of the I/O curve, there was a small decrease in MEPs (130%: -0.3 ± 0.52 mV). In addition, there was a small decrease in the whole level of CSE, as the I/O curve average reported (figure 20, -0.05 ± 0.14 mV). On the other hand, the NORM group showed a bigger decrease in the CSE, especially at higher stimulation intensities (110%: -0.18 ± 0.16 mV, 120%: -0.69 ± 0.92 mV; 130%: -0.08 ± 1.41 mV; 140%: -0.16 ± 0.88 mV). Surprisingly, however, the measure of the general excitability of the corticospinal tract reported an increase of the MEPs after the last training (0.09 ± 0.13 mV).

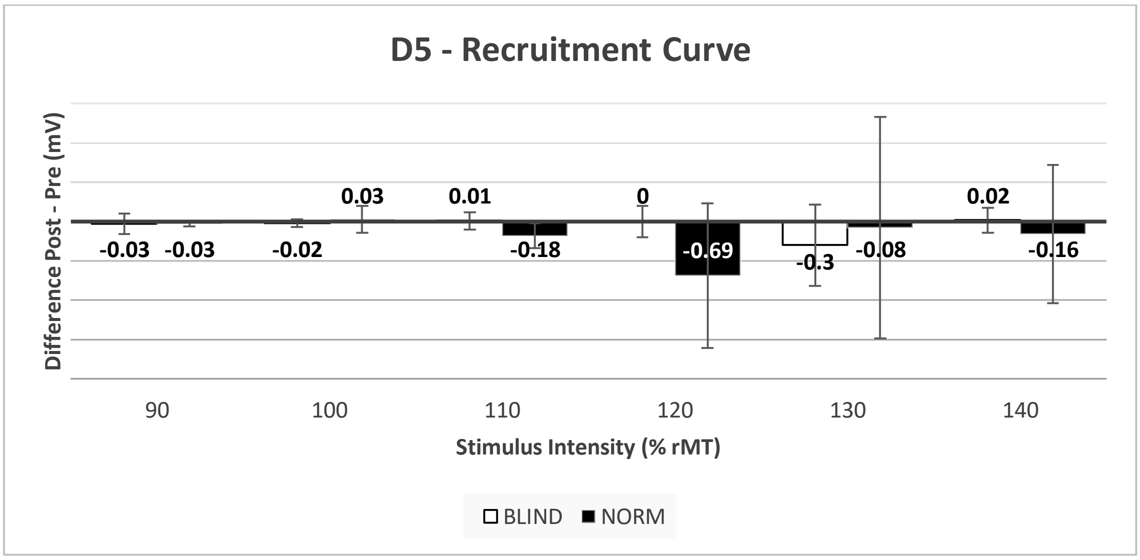


FIGURE 19 - The figure shows the difference between the post and the pre values for each intensity of the IO curve on day 5. The positive values represent an increase of the CSE in the post measurement. Conversely, when the value is negative, the CSE decreased in the post measurements.

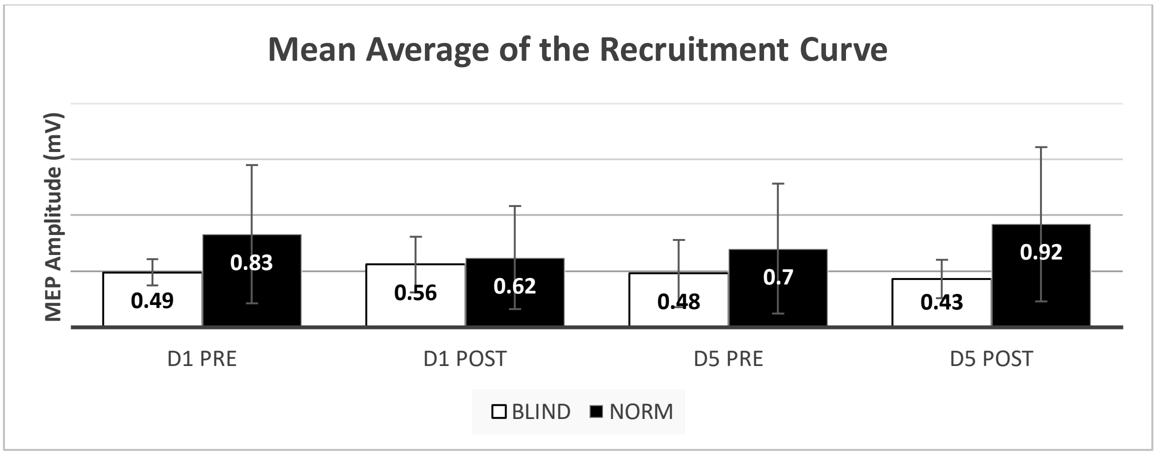


FIGURE 20 – The figure shows the overall excitability, obtained by averaging the IO curve intensities.

7.2.2 – SICI

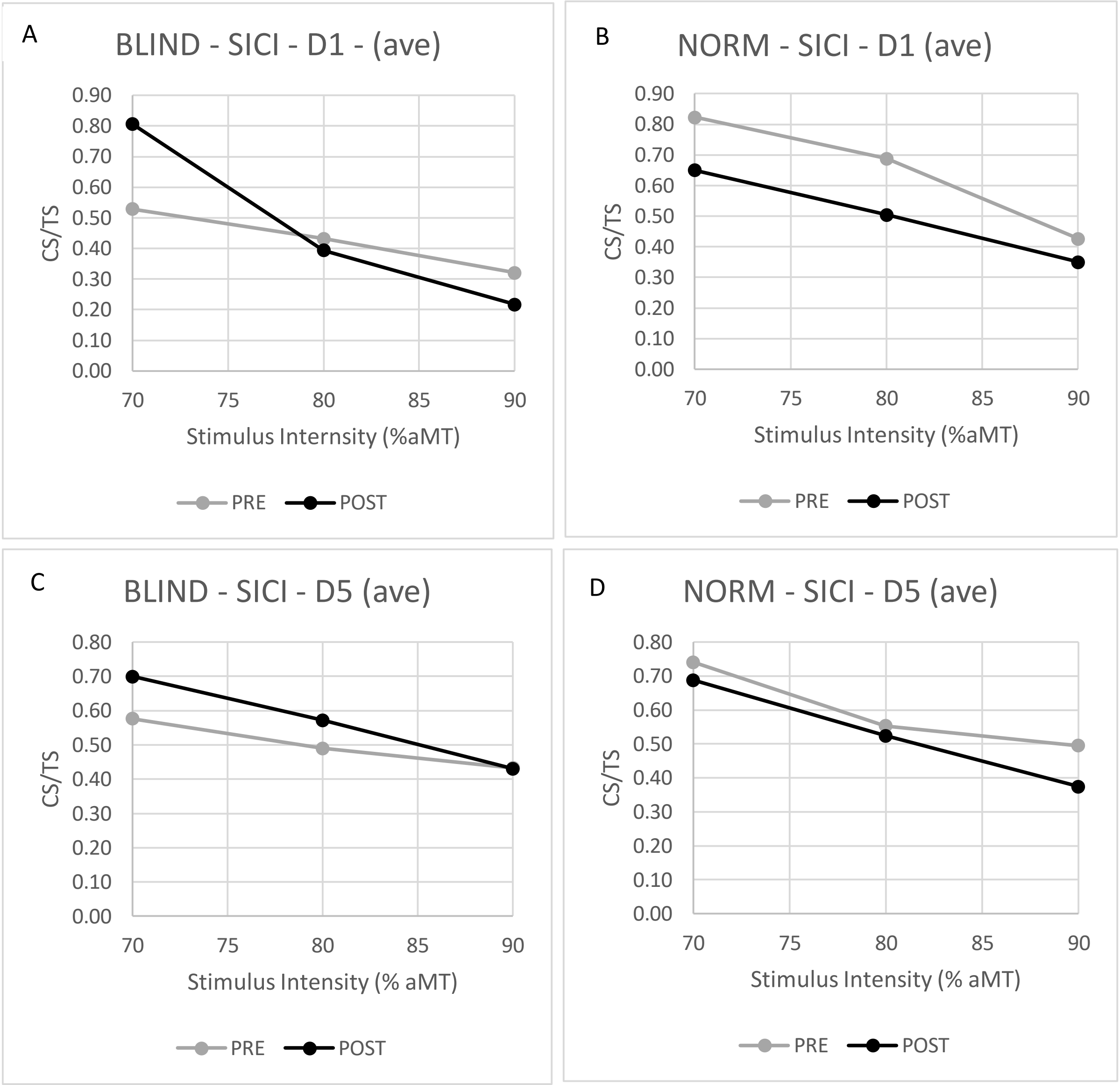


FIGURE 21 – The absolute values of the SICI curves of the two groups. The figures A and C depict the SICI curves of the BLIND group in D1 and D5, respectively; B and D represent the SICI curves of the NORM group on D1 and D5, respectively. The PRE and POST curves represents the measurements performed prior and after the practice, respectively.

Day 1. Measures of SICI, depicted on figure 22, suggest that on D1, the first training produced a slight decrease in the inhibition level for the BLIND group, as suggested by the dynamics of the SICI curve (the details are explained later in the text), and by the average of the whole curve (0.05 ± 0.14 mV, fig. 24). Although the higher conditioning stimulus intensities of the SICI curve showed a small increase in the inhibition (80%: -0.04 ± 0.26 mV; 90%: -0.1 ± 0.13 mV), at lower conditioning stimulus intensities there was a substantial decrease in the inhibition (70%: 0.28 ± 0.54 mV). Taken all together, it can be concluded that for the BLIND group, the first training induced a slight decrease in the inhibition of the CSE. On the other hand, the NORM group showed a pronounced increase in the inhibition level. Throughout the curve's stimulation intensities, the post-training conditioned MEPs were lower compared with the pre-training values (70%: -0.17 ± 0.10 mV; 80%: -0.18 ± 0.26 mV; 90%: -0.80 ± 0.23 mV). In addition, the overall measure of the cortical inhibition suggested a negative effect of the first training on the conditioned MEPs (-0.14 ± 0.11).

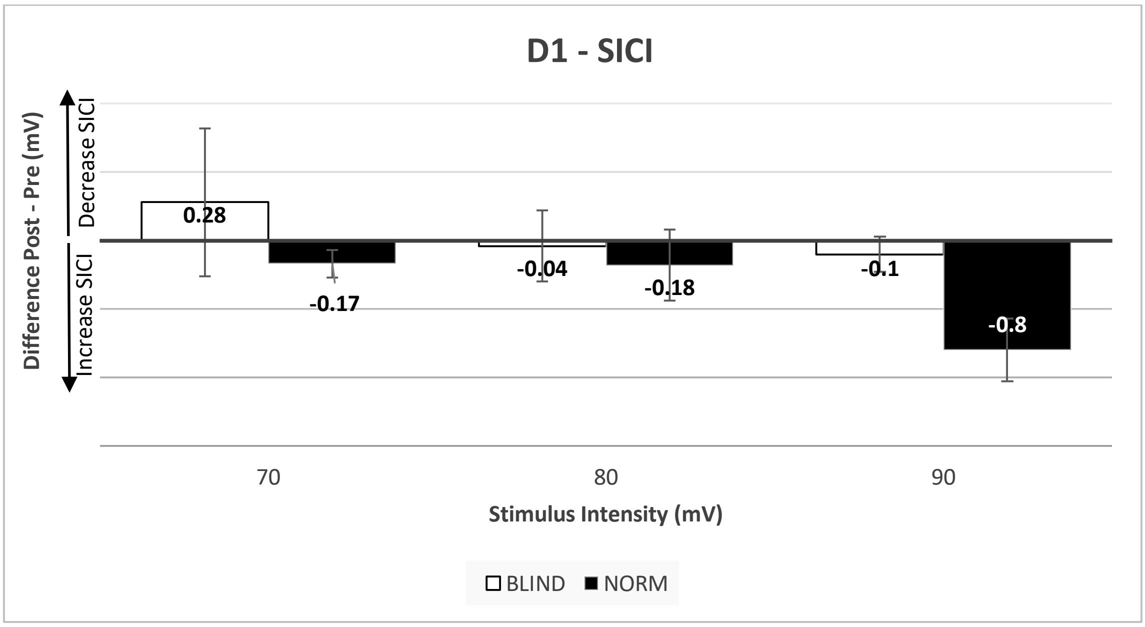


FIGURE 22 – The figure shows the difference between post and pre values of the SICI measurements for day 1. The SICI was define as the ratio between the conditioned and the unconditioned MEPs. A negative value represents an increase in the inhibition level, while on the contrary, a positive number represents a decrease in the inhibition level.

Day 5. On D5, the BLIND group showed a decrease in the intracortical inhibition after the last training, as figure 23 shows. This was suggested by the fact that the difference POST-PRE of the SICI curve was positive for each of the conditioning stimulus intensities, suggesting an increase in the amplitude of the conditioned MEP. In addition, the mean average of the SICI curve reported a slight increase in the overall excitability (0.05 ± 0.16 mV). On the contrary, the NORM group showed an increase in the inhibition levels, as shown by a decrease in the post-training conditioned MEPs values (70%: -0.08 ± 0.1 mV; 90%: -0.1 ± 0.45 mV). An increase in the cortical inhibition was suggested also by the mean average of the conditioned/unconditioned MEPs values, which reported a decrease of this parameter (-0.05 ± 0.16 mV).

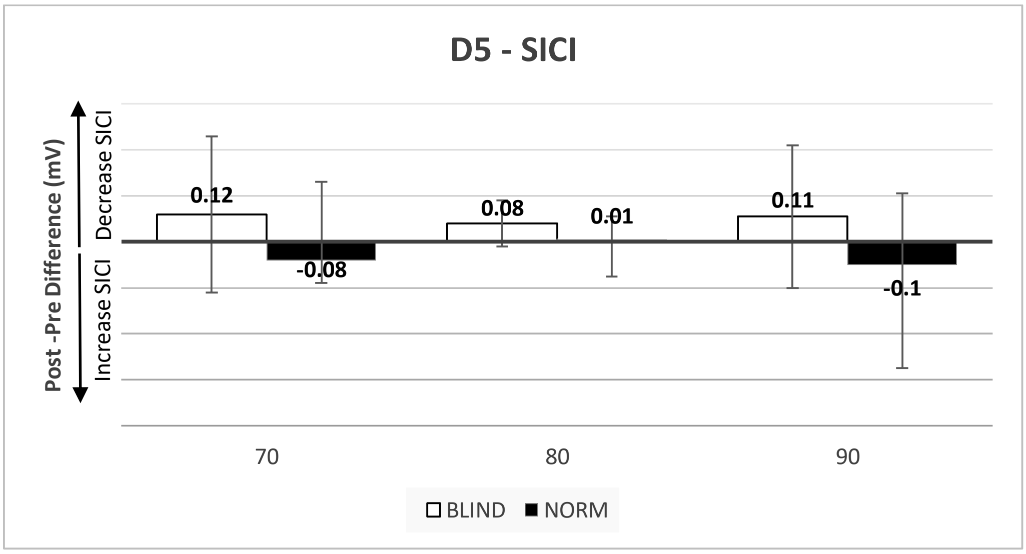


FIGURE 23 – The figure shows the difference between post and pre values of the SICI measurements for day 5. The SICI was define as the ratio between the conditioned and the unconditioned MEPs. A negative value represents an increase in the inhibition level, while on the contrary, a positive number represents an increase in the inhibition level.

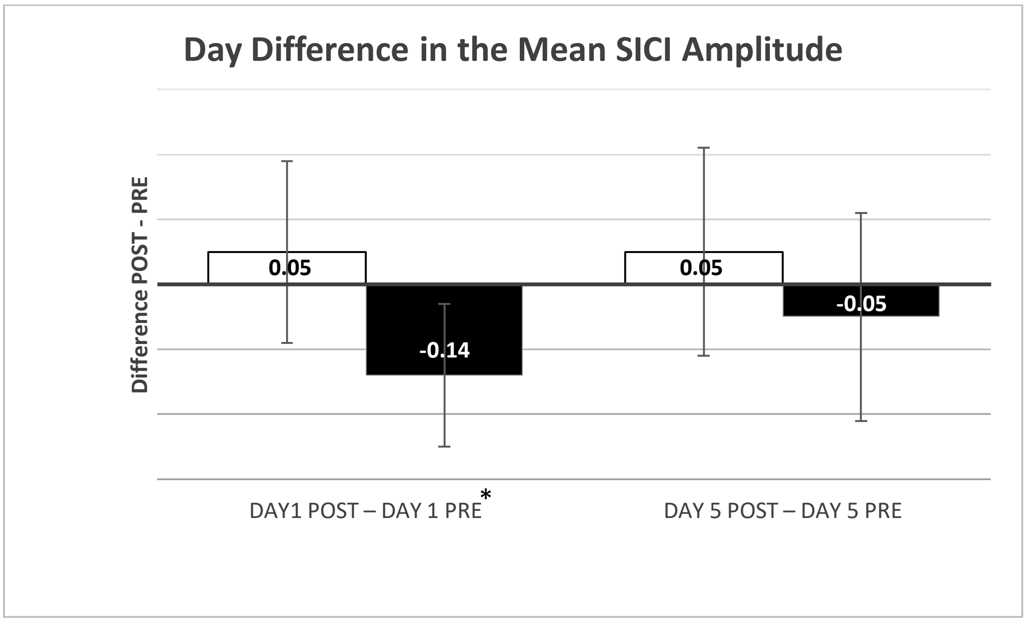


FIGURE 24 – The overall level of cortical inhibition was obtained by averaging the values from the various CS tested, that is the SICI curve. * $p < 0.05$.

7.3 – Performance and Motor Learning

Figure 25 shows the performance of the two groups throughout the 5 days of the learning program. The values represent the mean error of the subject's trials compared with the target line set by the researchers. The negative values represent a lower displacement of the cursor compared with the target line. On the contrary, when the values are positive, the displacement was increased. Thus, both positive and negative values represent an error in the performance.

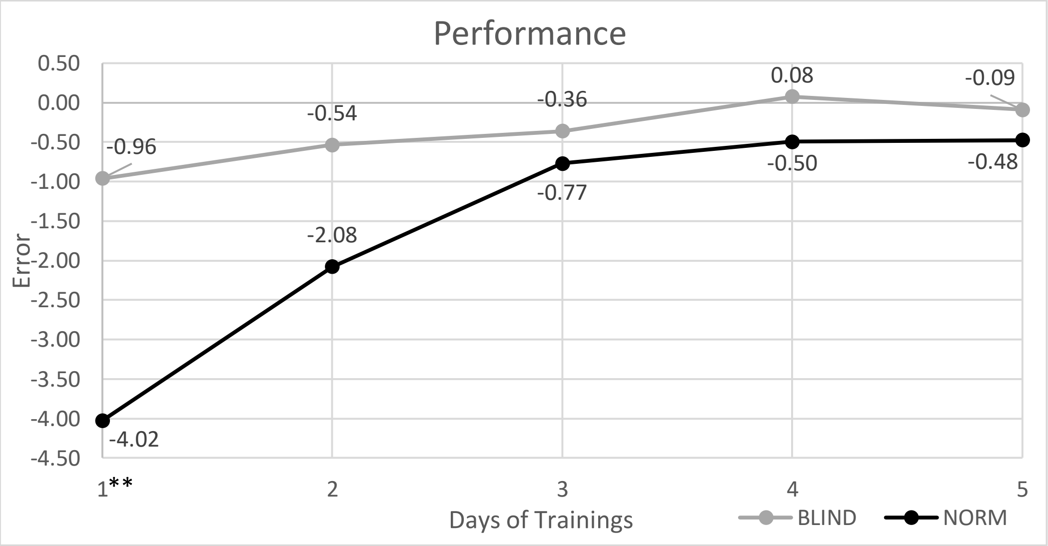


Figure 25 – The daily performance was obtained by averaging the trials performed on the various days. Both positive and negative values represent an error. The perfect performance is 0. ** $p < 0.01$

When the two groups are compared, the BLIND group performed better throughout the whole training program. There was a strong significant difference between the groups on D1 (Group difference 3.07 ± 1.17, $p < 0.01$). As the training progressed, this difference decreased and failed to report significance. However, the BLIND group performed always better than the NORM group.

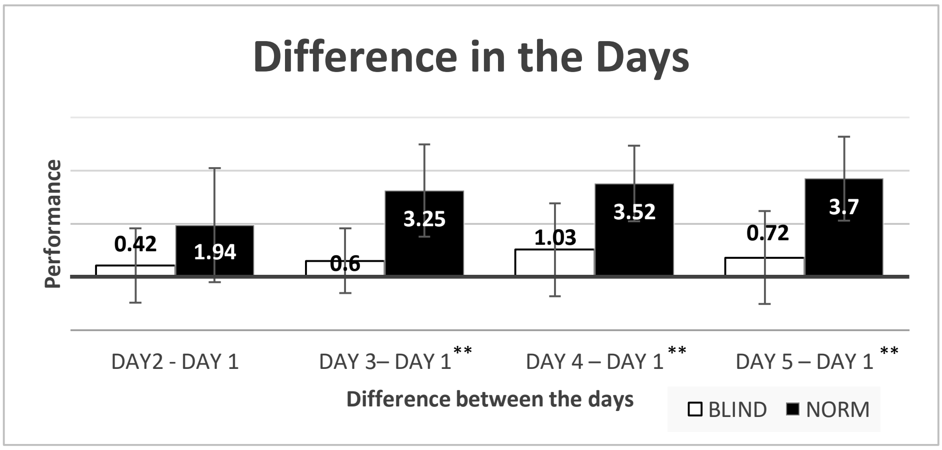


FIGURE 26 – The figure shows the effectiveness of the program, that is the difference in the performance between the training days and D1. Note that the two groups had different learning behaviours.

The difference between the days was calculated in order to assess the effectiveness of the training program. This difference gives insights about the learning rate throughout the training program. Figure 26 shows a graphical report of this difference in the performance throughout the program. The BLIND group did not show any significant difference in the performance throughout the days. On the other hand, the NORM group showed a strong significant difference when D1 was compared with D3 (3.25 ± 1.74, $p < 0.01$), D4 (3.52 ± 1.42, $p < 0.01$) and D5 (3.70 ± 1.59, $p < 0.01$). The difference between D1 and D5 represents the overall improvement in performance with the training. The BLIND group's improvement is small (0.72 ± 1.75) compared with the NORM group (3.70 ± 1.59), sign that the BLIND group was able to provide a better performance since the beginning.

8. DISCUSSION

8.1 – Behavioural Dynamics of the Blindfolded Training

The present behavioural results suggest a clear and unarguably positive effect of the blindfolding training on the performance. The BLIND group performed better than the NORM group since D1 (with a strong significant difference on D1 itself), and this increase in the performance was evident throughout the 5 days of the training. Noteworthy, the performance of the BLIND group on D1 was surpassed by the NORM group only on D3 (D1 BLIND: -0.95 ± 1.66, D3 NORM: -0.77 ± 1.80).

It is interesting to note that the NORM group's learning was larger than the BLIND group's one. This, however, is to be expected given the differences in the performance levels during the first days of training. Nevertheless, the increased rate of learning for the NORM group, does not affect the considerations of the effectiveness of the blindfolded training. In fact, the rate of learning is only a measure of the improvement in the performance. Usually, the higher this parameter is, the more effective a specific training is considered to be. However, the present study suggests that in some cases, having a lower rate of learning, is also positive. In fact, a high rate of learning level means, as it is the case of the NORM group, that at the beginning of the training program, the performance is not optimal, and efforts have to be made in order to improve it. On the other hand, with specific practices, the performance level can be brought to more advanced levels since the beginning of the training, leaving room for focussing on the details of the performance later in the training. It is the dream of every trainer, in every aspect of the motor learning realm, to have the trainee that do not improve much, because their performance is already high. Of course the consideration that a lower rate of learning is positive for the performance can be applicable only in the case of a high level of performance, and specific cases only.

How is that the simple act of blindfolding results in this dramatic difference in performance? In literature, it has been suggested that, among all, important components affecting the learning process are the type of instruction and the focus of attention (Wulf et al. 2010; Wulf 2007). In particular, it has been showed that the learning process sees a detriment if the learner focuses his attention on internal factors compared with external factors (Wulf et al. 1999). It is important, how those two types of attentional foci are defined. Wulf (2007a, pp.120) defines internal attentional focus as the "*attention directed to the action itself*", in contrast to the external attentional focus "*where the performer's attention is directed to the effect of the action*". At a superficial analysis, the attentional focus used in the present study could be considered as "internal". Under this logic, the present results seem to contradict the data reported in previous literature. However, the type of focus used in the present study does not match completely the definition given by most of the studies on this topic (Reviewed in Wulf 2007b). In fact, the instruction in this study was to pay attention on the proprioceptive information, in the form of "where is your body". On the other hand, studies on the different attentional foci, have interpreted the internal attentional focus as to "pay attention on the movement per se" that is to focus the attention on the posture or other internal factors, while paying any or little attention to the outcome (Wulf et al. 2010). In our study, the instruction was to try to use the proprioceptive information in order to increase the subject's body awareness. No instructions were given on which posture the subjects should have had, apart from "being comfortable".

On a purely speculative fashion, it is possible that the combination of the instruction, in the middle between an "internal" and "external" focus of attention (According to Wulf 2007, pp. 120), and the effects of the blindfolding (discussed later in the text), could have induced a higher level of performance.

8.2 – What happens when you blindfold yourself?

Leon-Sarmiento et al. (2005) suggested that 30 minutes of blindfold, either with eye open or closed produced an increase in the CSE, along with a decrease in intracortical inhibition. In that study the blindfold was applied for 30 minute continuously. The present study expands the results from Leon-Sarmiento et al. (2005). Indeed, the purpose of the BLIND measurements was to investigate the short-term dynamics of the corticospinal excitability when a certain pattern of blindfolding and non-blindfolding was applied. Specifically, the results of the present study show that as little as 15 seconds of blindfolding are enough to produce a small increase in the excitability of the corticospinal neurons, and this increase is extended for the 15 seconds after the blindfold removal. In other words, when a subject blindfold himself, a short-term neural mechanism increases the corticospinal excitability. One possible candidate for this positive effect of the blindfolding seems to be the action of GABA-ergic inhibitory interneurons (Reynolds & Ashby 1999; Di Lazzaro et al. 2004), which have been suggested to be a good index reflecting the general cortical excitability (Rosenkranz et al. 2007). 15 seconds of blindfold increased the SICI values (more precisely the ratio between the conditioned and unconditioned MEPs), a sign that is usually interpreted as decrease in the inhibitory level of the corticospinal neurons (Ilić et al. 2002). When the blindfold was removed, however, this disinhibition was reversed. In line with the increase in the inhibition level, the amplitude of the MEPs 15 seconds after the blindfold removal decreased.

In the present study, the motor task lasted 15 second. Therefore, we chose to limit the feedback to a maximum of 15 seconds. There was uncertainty on whether this timing of transient visual deprivation could have produce any change on the corticospinal excitability, as the ratio between the timing of the blindfolding vs non-blindfolding was 1:1. Surprisingly however, the results of the "BLIND measurements" suggest that this effect is indeed in place. In fact, each of the 3 10-minutes blocks of blindfolding pattern produced an increase in the corticospinal excitability when compared with the PRE measurements. This increase held up to the following 15 seconds of visual allowance.

The increase of MEPs was not combined, however, with a disinhibition of the corticospinal tract. On the contrary, Measures of SICI reported an increase in the cortical inhibition. In literature, multiple authors have shown that an increase in MEPs is usually accompanied with a decrease in cortical inhibition, and vice versa (Kujirai et al. 1993; Karin Rosenkranz & Rothwell 2003; Rosenkranz et al. 2007). So, the present results seem to contradict the results previously reported in literature. It could be argued that this increase in inhibition could be due to a state of sleepiness. However, in this state the cortical excitability has been reported to be lower than the awakening state. (Salih et al. 2005) Our result, in contrast, have reported an increase in the MEP size. It is therefore logic to exclude the sleepiness as possible cause of the increase of cortical inhibition.

Although there is a consensus that SICI is linked to measures of MEP, some authors have reported that in some cases, this interaction is not in place. For example, it has been recently shown that increasing the test MEPs, does not influence SICI measures, while seems to decrease long-interval intracortical inhibition (LICI, Opie & Semmler 2014).

Whether the increase in MEPs amplitude are due to a modulation of the levels of cortical inhibition, remain unclear. SICI, in fact is only one of a plethora of neurophysiological measures (Rossini et al. 2015). In the present study, this was the only measure that could have been measured, due to technical and time limitation.

8.3 – What Happens when you train blindfolded?

The present study investigated the MEP recruitment curve of a multi-joint movement but measured at the biceps brachii. It has to be admitted that the literature suggests that when a multi-joint movement is the focus of the study, other methods seem more suitable for the assessment of the corticospinal excitability. One of those methods is the TMS mapping technique (van de Ruit et al. 2015). Due to technical limitation, we were not able to use this technique. However, although sub-optimal, the I/O curve was shown to provide useful information on the CSE even in studies on motor learning with multi-joint movements. For example, Tyč & Boyadjian (2011) studied the corticospinal changes associated with 6 weeks of darts playing, and assessed both the I/O curve of the shoulder and brachioradialis, and the MEP amplitude map. They showed that after 6 weeks, the I/O curve of both muscles shifted toward an increase in the CSE, along an expansion of the TMS neural map (Tyč & Boyadjian 2011). Unfortunately, however, TMS studies on learning multi-joints movements are rare, with most of the studies focussing on single-joint or single-muscle movements (Rosenkranz et al. 2007; Perez et al. 2004; Kumpulainen et al. 2014).

Keeping in mind this limitation, this study provides interesting results nonetheless. Specifically, it is interesting to note the different behaviour of the I/O curve in the two groups on D1. The I/O curve saw an increase in the post-training for the BLIND group, whilst decreasing in its post-training values for the NORM group. This is consistent with the results of the BLIND measurements, which suggest a positive effect of transient visual deprivation on the corticospinal excitability. It has been reported that during a motor learning program, the neural correlate of the so-called “fast learning”, that is the within-session improvement of the performance, is suggested to be an unmasking of pre-existent neural connections (Pascual-Leone et al. 1995). This, along the reduced action of the GABA-ergic interneurons might account for the small increase in the CSE for the BLIND group (Coxon et al. 2014).

The methodological difference between the two groups was only in the blindfold application. Even the feedback and the encouragement were given in the same way. For both groups, the instruction was to focus their attention on the proprioception, rather than think on what to do next or in the relative movements of body parts with respect of the performance (Wulf 2007, pp. 120).

A good candidate for the explanation of the results on D1 is the role of attention. Indeed, it has been recently shown that attention on proprioceptive feedback has a positive effect on the CSE (Rosenkranz & Rothwell 2012). In addition, Mon-Williams et al. (1997) has shown that when the visual stimuli are not present or poor, the proprioception can have the dominance in the perception of the limb (see chapter 2 for more details). It is therefore possible that because the NORM group had normal visual stimuli, on the contrary to the BLIND group, this may have split their attention between the visual and proprioceptive stimuli. In other words, because the BLIND group did not have visual stimuli at all, they could concentrate better on the task. This could explain as well the fact that the BLIND group had a strong significant difference in the performance levels in the first days. In fact, researches have reported that when the attention is divided between different sources, the performance is affected (Scheerer et al. 2016).

A really intriguing, and somehow unexpected result is the fact that on D1, the NORM group, which was the control group in the present study, showed a decrease in the CSE. This is in contrast with previous studies on motor learning (Rosenkranz et al. 2007; Tyč & Boyadjian 2011), which showed that regardless on whether the training is done blindfolded or not, an increase in the corticospinal excitability is reported. As stated before, however, most of the studies have been performed on single-joint or single-muscle movements, and major differences in the control of those movements, in contrast to multi-joint movements, can be argued (Scott 2000). In addition, the task used in the present study was particularly challenging, in the sense that not only lasted 15 second, but was also, according to the definition of Schmidt & Wrisberg (2008, pp. 4-9), a continuous task (see table 1), that is all the movements were linked together in a sequence. This task is in clear contrast to the tasks used in other studies (For example the thumb abduction used by Rosenkranz et al., 2007). This might be a major difference, and in opinion of the author, could partly explain why for the NORM group, the IO curve decreased on D1. In fact, it can be argued that single-joint, movements are usually designed to maximise the effect of a certain manipulation on the neural correlate of the muscle controlling that joint.

Our task, on the other hand, could be argued to be the opposite. In fact, not only the task required a complex, multi-joint series of movements, but also required the subject to be constantly engaged with the whole training process, as they were not given on-line feedback. This could have spread the pattern of activation not only on the biceps brachii projection neurons, but to projection of other muscles as well, and this change would have been impossible to detect with our set up. Lastly the length of the task could have played an important role in the modulation of the corticospinal excitability, as could have resulted in an increase in the contribution of the premotor areas, which have been shown to be involved in action understanding, movement preparation, and complex movement control (Kandel et al. 2013, chapter 19 and 38 for a great reviews on the topic) In addition, it has been shown that the premotor areas, specifically the so-called mirror neuron system is activated during motor imagination (Kosslyn et al. 2001). A speculative model for the blindfold training including the mirror neuron system will be discussed in the next section.

On D5, the BLIND group showed little difference in the post-training values, as well as a slight decrease in the overall excitability. This is in line with the suggestion of Rosenkranz et al. (2007), that the later phase of the learning process is to be attributed to synaptogenesis, that is the creation of new cortical connection. In their study, Rosenkranz et al. (2007) reported that on D5, both the IO curve and the overall excitability did not show significant changes in their values. Measures of PAS, however, suggested that new synapses are created via LTP-like mechanism (Ziemann et al. 2001). The NORM group, on the other hand, reported a slight decrease in the post-training IO curve, as well as a slight decrease in the SICI measures. Interestingly enough, however, the overall post-training excitability was increased.

8.4 –Is the Blindfolded Training beneficial? A speculative model.

During the writing process of this manuscript, the author has been asked several times questions like “Why would you perform a training blindfolded?” or “What is the beneficial for the training itself, and not only for the performance?”. At this point, all the possible explanations are largely speculative, due to a lack of studies addressing this issues, as mentioned before. However, if we step back from the analysis of only M1 (which was the stimulated area in this study, and therefore the only area for which an in-depth analysis was possible), and we enlarge our analysis on the premotor areas as well, it is possible to provide a model that, although mostly speculative, fits very well the present results and the concept of the blindfolded training as well.

Leon-Sarmiento et al. (2005) suggested that the modulation of the corticospinal excitability following 30 minutes of visual deprivation, either blindfolded or not (in the dark, however) could be due to an “unmasking of the occipito-frontal projections, mainly from the areas F4 and F5. Those areas, also called “mirror neurons system”(Cattaneo & Rizzolatti 2015), are involved not only in the motor function, but also in action observing. In primate experiments, those areas fired in a similar way not only when the monkey performed a certain action, but also when the action was performed by another person, and the monkey observed (Fogassi et al. 2005). Interestingly, however, those areas have been suggested to be involved also in motor imagery (Kosslyn et al. 2001). Although the present study did not address motor imagery as such, it is undeniable that the blindfolded subject could have used the imagination in order to “picture the trajectory”. A similar conclusion was also suggested from studies on mental object rotation (Richter et al. 2000). Kosslyn et al. (2001) suggested that in the case of mental object rotation, two possible strategies could have been used. The first one involves to “*imagine what the subject would see if he manipulated the objec*t”, while the other would involve “*what the subjects would see if someone else would manipulate the object*”. The first strategy could have relevance in the concept of the blindfolding training. In fact, it has to be noted that the BLIND group was completely “blind” during the trial, and that, as for the NORM group as well, they receive off-line feedback between the trials. Therefore, it can be speculated that in those conditions, they could have created a "mental map" of the trajectory. This along with an increase attention posed to the proprioceptive feedback, could have had a facilitatory effect of the corticospinal neurons, and therefore an increase corticospinal excitability. This could also explain why the BLIND group performed significantly better than the NORM group in the first part of the training program.

The model presented, as mentioned earlier, it is mostly speculative, as we did not measure the contribution of the premotor areas to the increase in corticospinal excitability. Future studies will have to address this contribution as well as expanding our knowledge in the physiology of the blindfolding.

8.5 – Conclusions

To the knowledge of the author, the present study was the first to address the question “Is a training program based on transient visual deprivation beneficial of the learning process?”. Basing the judgement on the performances that the 2 groups have produced, it is unarguably that the blindfold training is beneficial for the learning process. It is however too soon, by the scientific standards, to state that this beneficial effect is universal and applied to all conditions. The novelty of this concept is of course naturally followed an uncertainty on the physiological and neural correlates behind this idea. The main conclusions of our study suggests that using this task, a training performed blindfolded produces an increase in the corticospinal excitability compared with the same training performed non-blindfolded. This modulation takes place as soon as 15 seconds after the blindfolding application, even in resting condition. The fact that the neurophysiological parameters measured in this study failed to show significance, does not necessarily means that the modulation of the corticospinal tract was meaningless. In fact, the sample size in the present study was small (BLIND: 4; NORM: 5 subjects). This might have affected the statistical process. However, looking at the performance results, it can be safely state that a modulation of some sort must have happened within the CNS, which our results did not fail to report.

Nevertheless, future studies are required to clarified the physiology of the possible mechanisms of this modulation, which to date remain uncertain. It seems plausible that the combined effect of an increased attention (by the removal of visual stimuli) on the proprioceptive signals and their better integration (sensorimotor integration) within the brain might be a good candidate. However, these results are not conclusive on what are the mechanisms responsible for this modulation.

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APPENDICES

Appendix 1 – The layers of the Neocortex (From Kandel et al. 2013, ch.16)

Layer	Type of Neurons	Function	Dendrites from other layers
Molecular (I)		This layer is formed only by the dendrites of deeper layers and axon traveling through this area to make connections in other cortical areas.	It contains apical dendrites that have the cell body in layer V and VI, as well as layer II and III
External Granular (II)	Pyramidal and Spherical neurons	The axons of the neurons in this layer project locally to other neurons, as well as to other cortical areas, mediating intracortical connection	It contains apical dendrites that have the cell body in layer V and VI, as well as layer II and III
External Pyramidal (III)	Pyramidal neurons	The axons of the neurons in this layer project locally to other neurons, as well as to other cortical areas, mediating intracortical connection	It contains apical dendrites that have the cell body in layer V and VI, as well as layer II and III
Internal Granular (IV)		It is the main recipient for sensory inputs from the thalamus and it is the predominant layer of the primary sensory areas.	
Internal Pyramidal (V)	Pyramidal neurons, larger than the ones in layer III	This layer give rise to the major output pathways of the cortex, projecting to other cortical and subcortical areas.	It contains the basal dendrites of the neurons with cell body in layer III and IV, as well as layer V and VI
Polymorphic (VI)	Various neurons	It blends with the white matter that form the deep limit of the cortex and carries axons to and from the cortex.	It contains the basal dendrites of the neurons with cell body in layer III and IV, as well as layer V and VI

Appendix 2 – Informed Consent and TMS screening

UNIVERSITY OF JYVÄSKYLÄ

University of Jyväskylä - Information sheet for the study participants

Name of the Study

The Effects of Transient Visual Deprivation on Motor Cortical Excitability.

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Set-up, Inclusion Criteria and Timetable of the Research

The purpose of the study is to assess the possibility that a skill training based on transient visual deprivation, through blindfolding, could produce a better motor learning from the neurophysiological point of view.

In order to quantify the motor learning, the primary motor cortex (M1) will be stimulated using Transcranial Magnetic Stimulation (TMS). This is a non-invasive method of brain stimulation, which uses magnetic stimuli to excite the cerebral cortex. It does so in a safe way for the subject, with little or limited discomfort (see later for safety details).

The skill training will consist of 5 training sessions of 30 minutes each, in which the participants will learn to perform a visuomotor task. The participants will be asked to move a handle connected to a pulley. The movement of the handle will produce a movement trajectory, which will be required to be as close as possible to the performance pathway set by the researchers.

The participants will be randomly divided in 2 groups, namely “NORM” and the “BLIND”. The NORM group will train for the whole time using visual stimuli, while the BLIND group will perform the part of training sessions blindfolded, and will be allowed to take off the mask only for feedback.

Inclusion Criteria

In the present study, no differences are made between male and female participants. On the other hand there are three major requirements, which will be used as inclusion criteria:

1. Being right-handed
2. Being Physically active
3. Not being highly skilful in sport in which the upper limb is part of the technique (i.e. table tennis, basketball, tennis, volleyball, floor-ball, etc.).

Before the measurements those 3 inclusion criteria will be assessed using specific questionnaires (see later).

Time Table

The participants will have the freedom to choose the period in which they will participate to the project, within the one set by the researchers (see the appendix). The times of the measurements and training will be agreed between the participant and the researches, considering the laboratory, the researcher and the participant's availability.

The timetable of the involvement for the participant is summarized below. The times are indicative and might have an excursion of 15-30 minutes.

Day 0 **Measurements** (Week before the training program)	**Day 1** **Measurements and First Practice** (Mon/Tue)	**Day 2 to Day 4** **Practice**	**Day 5** **Last Practice and Measurements** (Fri/Sat)
2 hr	3 hrs	30 min	3 hrs

Before the beginning of the training week, they will be asked to participate to a familiarization and preliminary measurements. These measurements will last around two hour. The next week, on day 1 (Monday or Tuesday) and day 5, the pre- and post-training measurements will be performed. These measurements will last around 3 hours. During the training week (day 2 to day 4) the subject will be asked to come to the laboratory for a short training of 30 minutes in each day.

Risks Associated with the Research Measurements

The present study does not present any explicit danger for the participant. The use of TMS has been reported to be safe for the subjects, with little or limited potential side effects. The table summarize the potential side effects of the use of TMS. After the use of TMS, the participant may experience light headaches and local pain. In rare and extreme cases, the participant can experience seizures or syncope.

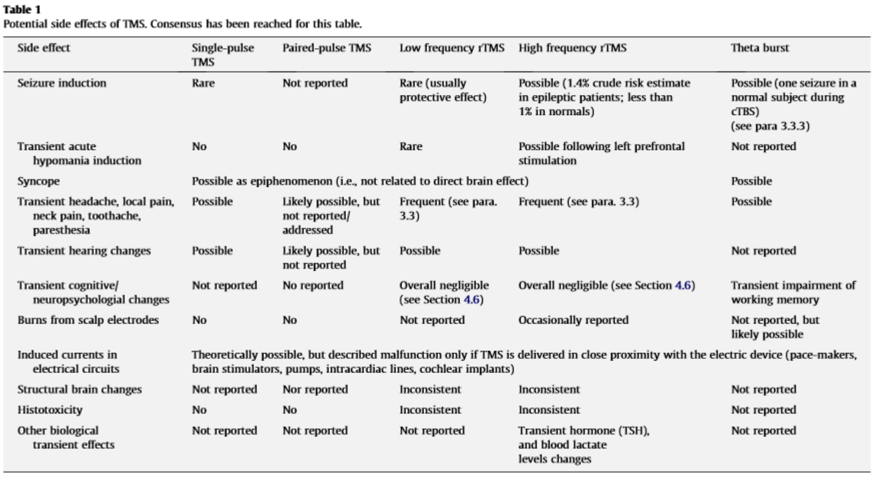


Table 1
Potential side effects of TMS. Consensus has been reached for this table.

Side effect	Single-pulse TMS	Paired-pulse TMS	Low frequency rTMS	High frequency rTMS	Theta burst
Seizure induction	Rare	Not reported	Rare (usually protective effect)	Possible (1.4% crude risk estimate in epileptic patients; less than 1% in normals)	Possible (one seizure in a normal subject during cTBS) (see para 3.3.3)
Transient acute hypomania induction	No	No	Rare	Possible following left prefrontal stimulation	Not reported
Syncope	Possible as epiphenomenon (i.e., not related to direct brain effect)				Possible
Transient headache, local pain, neck pain, toothache, paresthesia	Possible	Likely possible, but not reported/ addressed	Frequent (see para. 3.3)	Frequent (see para. 3.3)	Possible
Transient hearing changes	Possible	Likely possible, but not reported	Possible	Possible	Not reported
Transient cognitive/ neuropsychologial changes	Not reported	No reported	Overall negligible (see Section 4.6)	Overall negligible (see Section 4.6)	Transient impairment of working memory
Burns from scalp electrodes	No	No	Not reported	Occasionally reported	Not reported, but likely possible
Induced currents in electrical circuits	Theoretically possible, but described malfunction only if TMS is delivered in close proximity with the electric device (pace-makers, brain stimulators, pumps, intracardiac lines, cochlear implants)				
Structural brain changes	Not reported	Nor reported	Inconsistent	Inconsistent	Not reported
Histotoxicity	No	No	Inconsistent	Inconsistent	Not reported
Other biological transient effects	Not reported	Not reported	Not reported	Transient hormone (TSH), and blood lactate levels changes	Not reported

Use of Research Results

The data and the results of the analysis of the data collected during the measurements will be used for writing a master thesis. In addition, the results might be presented in conferences and published in international journals. The results will be presented in an anonymous way, with no personal details provided that might indicate the identity of the participant. The participant can ask to know his/her own results, once the measurements are completed.

Rights of Participants

The Participation is completely voluntary. The participants can withdraw at any point of the research period, without any consequence. As stated before, the data will be treated confidentially, and no details will be shared with other people apart from the research group. In addition the participant has the right to ask at any time further information about the confidentiality of his/her data.

TMS SAFETY QUESTIONNAIRE

Please answer to the following questions:

1. **Do you have epilepsy or have you ever had a convulsion or a seizure?**

YES NO

2. **Have you ever had a fainting spell or syncope?**

YES NO

If yes, please describe on which occasion: ______________________________________

3. **Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?**

YES NO

4. **Do you have any hearing problems or ringing in your ears?**

YES NO

5. **Do you have cochlear Implants?**

YES NO

6. **Are you pregnant or is there any chance you might be?**

YES NO

7. **Do you have metal in your brain, skull or elsewhere in your body? (e.g. splinters, fragments, clips, etc.)?**

YES NO

If so, specify the type of metal: ____

8. **Do you have an implanted neuro-stimulator (e.g. DBS, epidural/subdural, VNS)?**

YES NO

9. Do you have a cardiac pacemaker or Intra-cardiac lines?

YES NO

10. Do you have a medication infusion device?

YES NO

11. Are you taking any medications?

YES NO

If yes, please list: ____

12. Did you ever undergo TMS in the past?

YES NO

If yes, were there any problems?_________________________________

YES NO

If yes, please describe which problems did you have: ________________

13. Did you ever undergo MRI in the past?

YES NO

If yes, were there any problems?

YES NO

If yes, please describe which problems did you have: ________________

Reference:

Rossi, S. et al., 2011. Screening questionnaire before TMS: an update. *Clinical Neurophysiology*, 122(8), p.1686.

Rossini, P.M. et al., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, 126(6), pp.1071–1107.

Notes:

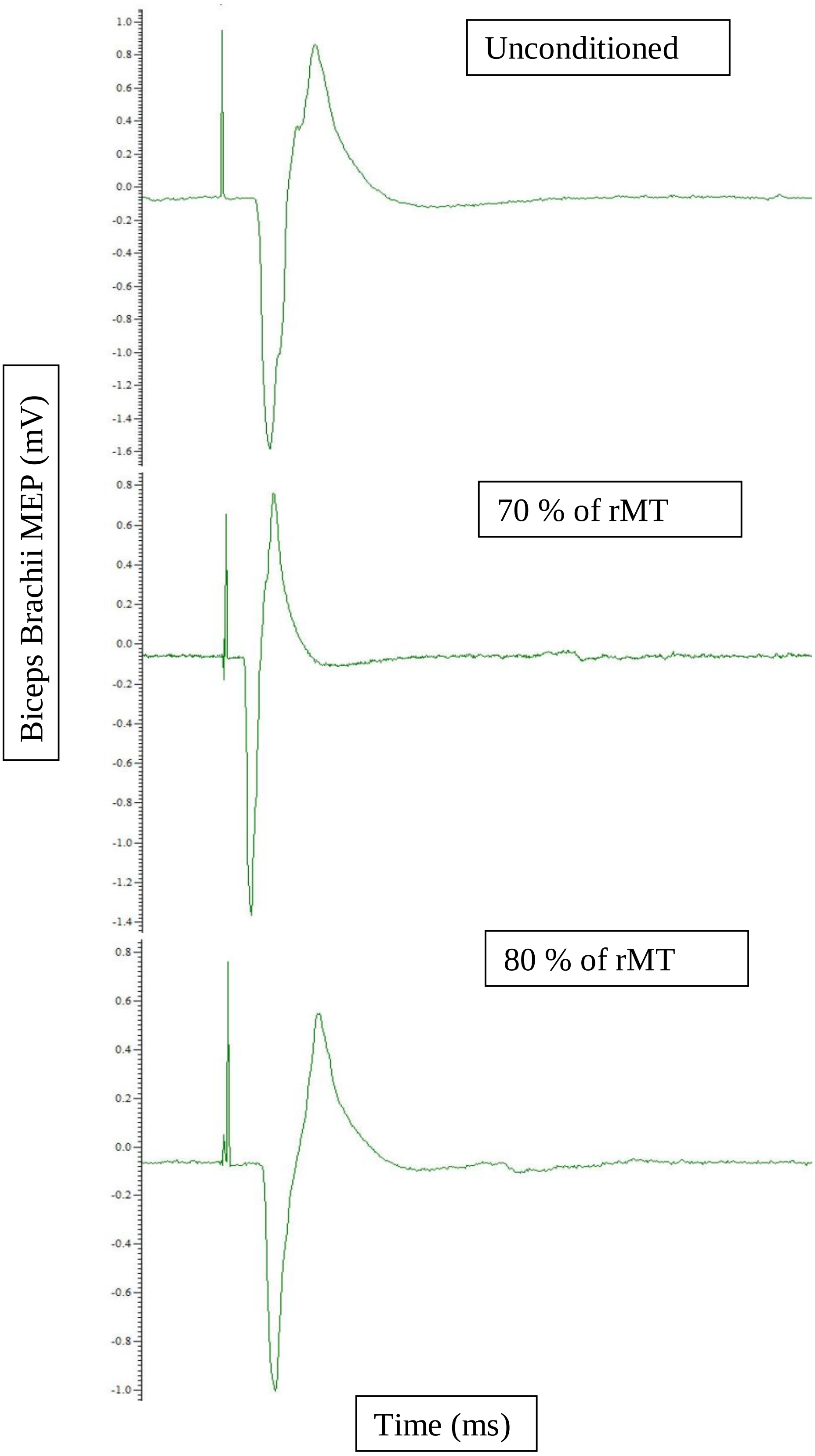
Date

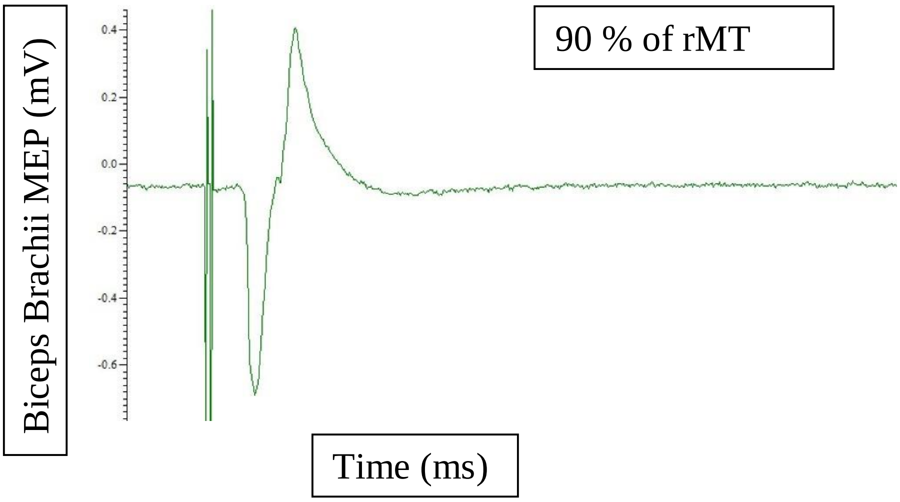
Name of the participant (capital letters) and signature

Name of the Researcher in charge (capital letters) and signature

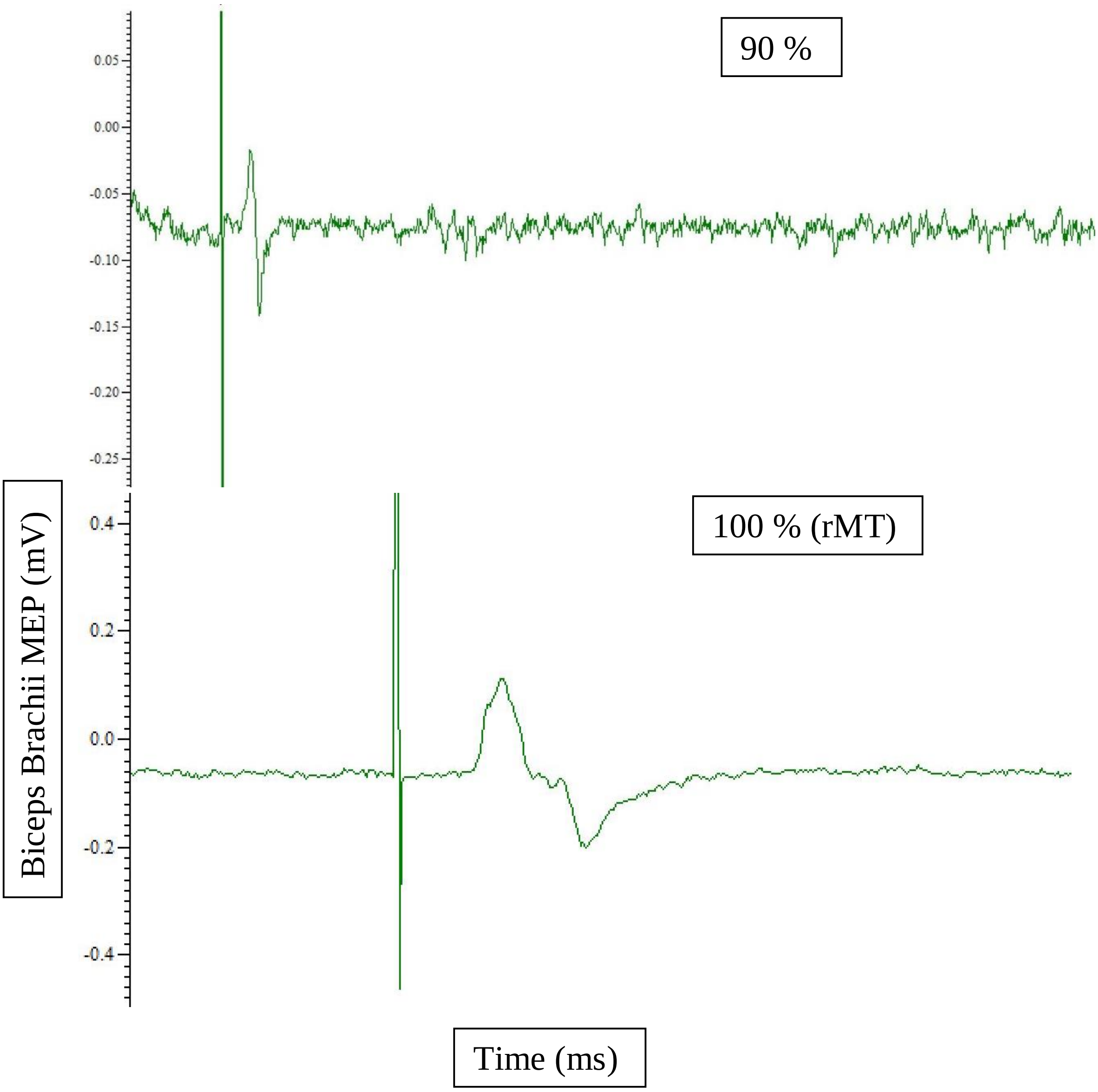
Appendix 3 – Raw Signals

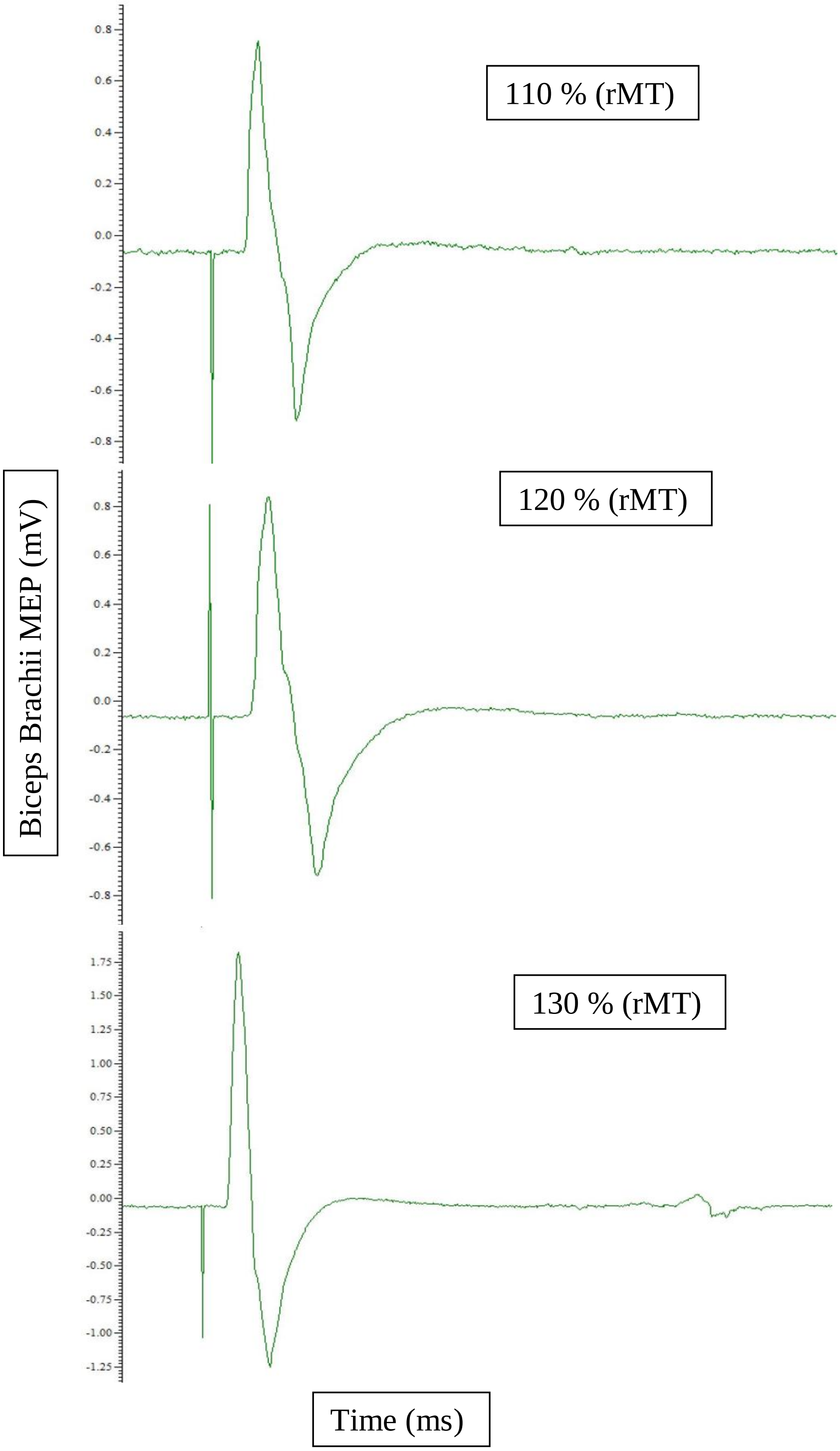
Short-Interval Intracortical Inhibition (SICI)

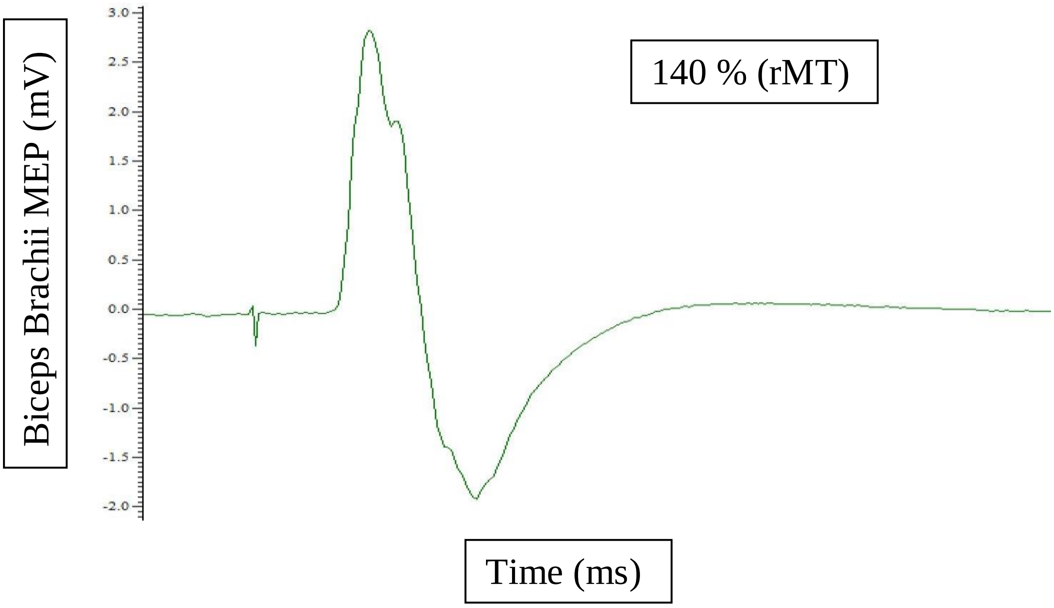




I/O Relationship of the MEPs




110 % (rMT)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
120 % (rMT)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
130 % (rMT)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
Biceps Brachii MEP (mV)
Time (ms)


Biceps Brachii MEP (mV)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
140 % (rMT)
Time (ms)