The Neuroplasticity Paradox: Can Lack of Neuroplasticity Protect Older Adults from Motor Memory Interference?

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TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

Abstract

Motor memory consolidation is a process by which motor memories encoded during the practice of a motor skill are transformed from an initial fragile state to a more solid and stable state (Widmaier et al., 2016). Motor memories encoded by older adults are easily disrupted when another task is practiced soon after practice, during the first stages of consolidation (Roig et al, 2014). However, the neural mechanisms underlying this higher susceptibility to memory interference in older individuals are not known. Transcranial magnetic stimulation (TMS) is a non-invasive stimulation (NIBS) technique that can be used to manipulate brain activity, thus providing insights into the mechanisms involved in memory formation processes (Censor et al., 2011). Previous studies with young adults have shown that the primary motor cortex (M1) is essential for the first stages of motor memory consolidation (Muellbacher et al., 2002). Furthermore, deficits in the consolidation of motor memory in older adults are commonly attributed to an impaired capacity to trigger mechanisms of synaptic plasticity such as long-term potentiation (LTP) during motor practice (Cantarero et al., 2013). TMS can be used to trigger LTP-like mechanisms but also its opposite -long-term depression (LTD)-, transiently reducing the excitability of cortico-spinal pathways (Rossini et al., 2015). In this study, after practicing a motor task, young and older subjects received low frequency repetitive TMS (rTMS) to induce motor memory interference and disrupt memory consolidation. Moreover, corticospinal excitability (CSE), a marker of synaptic plasticity in cortico-spinal pathways, was assessed in specific time-points after practicing the motor task. Skill retention was assessed 8h and 24h after motor practice to investigate differences in sleep-dependent and non-sleep dependent consolidation processes between groups. Overall, no statistically significant differences in skill retention or in the CSE time-points were found between groups. However, older subjects that were less susceptible to the immediate effects of the rTMS improved more so the response time of the sequence during the non-sleep dependent consolidation. Moreover,

with the CSE normalized to the baseline, older subjects who were capable of recovering from the interfering effects of rTMS improved more the response time of the sequence of the second retention in comparison with the first retention. In summary, paradoxically, the older subjects that had a lack of plasticity in M1 were capable of creating new connections with other brain areas during consolidation were the ones that had better results.

Résumé

La consolidation de la mémoire motrice est un processus par lequel l'encodage de nouvelles habiletés motrices, suite à leur pratique, passe d'un état initial fragile à un état plus stable et solide (Widmaier et al., 2016). Chez les personnes âgées, la consolidation d'une habileté motrice récemment encodée est facilement perturbée par une seconde tâche qui est pratiquée lors des premières étapes de sa consolidation (Roig et al, 2014). Toutefois, les mécanismes neuronaux reliés à la plus grande sensibilité de la mémoire aux interférences observée chez les personnes âgées ne sont toutefois pas identifiés. La stimulation magnétique transcrânienne (TMS), une technique de stimulation non invasive (NIBS), peut être utilisée pour moduler l'activité cérébrale et ainsi fournir un aperçu des mécanismes impliqués dans la formation de la mémoire (Censor et al., 2011). Des études antérieures ont démontré que le cortex moteur primaire (M1) est essentiel pour les premières étapes de la consolidation de la mémoire motrice chez de jeunes adultes (Muellbacher et al., 2002). De plus, les déficits dans la consolidation de la mémoire motrice chez les personnes âgées sont associés à une altération au niveau de l'induction des mécanismes de plasticité neuronale durant la pratique motrice telle que la potentialisation à long terme (LTP) (Cantarero et al., 2013). La TMS peut être utilisée pour déclencher des mécanismes similaires à la LTP ou à son opposé -la dépression à long terme (LTD)- qui réduit transitoirement l'excitabilité des voies cortico-spinales (Rossini et al., 2015). Après avoir effectué une tâche motrice, les deux groupes (jeunes et vieux) ont reçu un protocole de TMS répétitif à basse fréquence (rTMS) afin d'induire une interférence dans la consolidation de la mémoire motrice et ainsi perturber la formation de la mémoire. L'excitabilité corticospinale (CSE) a été mesurée à des moments précis durant l'heure suivant la tâche motrice et sa rétention a été évaluée 8 et 24 heures après l'encodage de celle-ci recherchant des différences dans les processus de consolidation dépendants et non dépendants du sommeil. Aucune différence statistiquement significative n'a été trouvée dans les compétences motrices

ou dans la CSE entre les deux groupes. Cependant, les participants âgés qui sont moins sensibles aux effets immédiats de la rTMS améliorent davantage leur temps de réponse mesurée avec une consolidation sans sommeil. De plus, en normalisant la CSE à sa valeur de base, les sujets âgés qui sont moins affectés par la rTMS sont ceux qui améliorent davantage leur temps de réponse lors de la deuxième rétention comparativement à la première rétention. En résumé, paradoxalement, les sujets plus âgés qui avaient un manque de plasticité en M1 ont été capables de créer de nouvelles connexions avec d'autres zones du cerveau pendant la consolidation et ont été ceux qui ont eu les meilleurs résultats.

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Preface

This Master's thesis was organized according to the guidelines of McGill University's Faculty of Graduate and Postdoctoral Studies.

This thesis is arranged in the following order:

Chapter I: Introduction: introduces the rationale and the objectives of the study

Chapter II: Background: presents a review of the literature of the area of study

Chapter III: Methods: provide a detailed description of the study methodology

Chapter IV: Results: presents the statistical results of the study

Chapter V: Discussion: provide a debate relating the results and the literature

Chapter VI: Summary and Conclusion: delivers the main finding of this study, its clinical significance and implication

References: list of reference and appendices

This thesis complies with McGill's policy of intellectual property and all ethical standards.

Contribution of Authors

Diogo Medeiros was responsible for the ethics application, data collection, analysis and writing of the thesis. Dr. Marc Roig supervised all the aspects and was also responsible for the design of the study, obtaining funding and intellectual propriety. Jean-Francois Nepveu was responsible for the recruitment of the subjects and data collection. Carla Centeno and Zhen Lun Chen participated in data collection.

CHAPTER I: INTRODUCTION

1.1 Relevance and Importance

Statistics Canada has shown that "The median age of Canada's population has grown by 10.2 years in the past 30 years […] the most recent projections show that by 2036, seniors could constitute more than one-fourth of the Canadian population." Aging is associated with a decline in certain cognitive abilities while others are kept intact (Brown et al., 2009) and, also, with the increase in the risk the of illnesses such as Alzheimer's disease (Lutz et al., 2008; Deary et al., 2009). Cognitive impairments will be accompanied, inevitably, by challenges to maintain mobility and thus could potentially limit elderly population's functional independence, also because older adults have difficulties in transform new motor skills into long-lasting memory (Brown et al., 2009). Although there has been scientific progress in the recent years allowing for a better understanding of the consequences of aging on cognitive abilities, the plethora of neural mechanisms potentially underlying the memory loss experienced by older individuals remain to be fully understood. Therefore, it is imperative to gain insights into the neurobiology and neurophysiology underlying age-related declines in memory; it could potentially lead to novel therapeutic interventions to prevent or slow down cognitive decline in the older population, thus allowing them to maintain functional independence (Freitas et al., 2011).

1.2 Rationale

Motor memory can be defined as the ability for one to replicate a motor skill and perform better during an ulterior trial of the task (Widmaier et al., 2016). Several studies have shown that elderly people show deficits in motor learning (Seidler et al., 2010). However, these deficits do not seem to affect all phases of motor learning to the same extent (Smith et al., 2005). Compared to young counterparts, motor learning in elderly subjects is normally

characterized by a lower skill level at baseline and a slower rate of improvement during motor practice. However, when normalized to baseline values, elderly subjects may show similar or even superior improvements during skill acquisition (Brown et al. 2009). In contrast, there is strong evidence that elderly people have deficits in the retention of motor skills once motor practice has ended (Brown et al. 2009; Roig et al. 2014; Centeno et al., 2018).

Successful motor learning depends, to a large extent, on the capacity to form strong motor memories that contain the sensorimotor information acquired during skill practice. Like other, more explicit types of memory (e.g. episodic memory), motor memories need to go through three main stages before they are formed: encoding, consolidation, and retrieval. Encoding (i.e. skill acquisition) is essential, because it is during this initial phase of the memory formation process that the nervous system receives the sensorimotor information that will later be used for the elaboration of motor memories. However, the brain does not stop processing the information after encoding it. It is through consolidation, a process that continues to evolve long after encoding, that a motor memory trace matures and is progressively strengthened. If consolidation is successful (Robertson et al., 2004a), the motor memory becomes more robust, less susceptible to be disrupted and ready for retrieval. This concept is especially important because if there is a perturbation during the consolidation phase of a motor memory (e.g. motor memory interference), the memory trace would not be consolidated properly and thus the capacity to retain the motor skill practiced previously would be impaired.

Interference has been well characterized behaviorally using combinations of different motor tasks practiced in succession (Robertson et al., 2004a). Cantarero et al., for example, demonstrated the motor memory interference effect using a two-day sequence experiment. Subjects were randomly divided into four groups: AA (learning of task A on day one, and retention of task A on day two), ABA (learning of task A followed by learning of task B on day one and retention of task A on day two), BAA (learning of task B followed by learning of

task A on day one and retention of task A on day two) and a control group assigned to do randomized versions of the task. The groups that learned a secondary (B) task, ABA and BAA, had a poorer performance compared to their AA counterpart, thus implicitly suggesting the consequences of interference on motor memory consolidation. We have extended these findings using an ABA paradigm to show that elderly individuals have an increased susceptibility to memory interference (Roig et al., 2014). Understanding how aging affects the susceptibility to motor memory interference is clinically relevant because most people in rehabilitation programs, aimed at motor recovery after brain injury (e.g. stroke), have an advanced age. Unfortunately, the neurobiological underpinnings underlying this age-related increased susceptibility to motor memory interference remain unknown.

Motor learning leads to long-term potentiation (LTP), a cellular process in which the connection between synapses tagged during memory encoding strengthens, thus facilitating the consolidation of memory (Rioult-Pedotti et al., 1998). Motor memory interference can thus be induced by suppressing LTP, which is a key process in the consolidation of the memory (Tunovic et al., 2014). One way to suppress LTP-like mechanisms in the brain is to induce long-term depression (LTD), which is and antagonist to LTP, by using non-invasive brain stimulation (NIBS) techniques such as transcranial magnetic stimulation (TMS). It has been seen that LTD-like plasticity relies primarily on the Gamma-Aminobutyric Acid -GABA-inhibitory system) (Hess and Donoghue, 1996). In the motor cortex, this LTD-like form of plasticity results in decreases in cortico-spinal excitability (CSE) (a marker of synaptic plasticity in motor cortical areas influenced by LTP-like mechanisms) (Huang et al., 2005). For instance, low frequency (1Hz) repetitive TMS (rTMS) was described to be effective in reducing the CSE of younger adults when compared to older adults (Todd et al., 2010). Indeed, because LTP (strengthening of synapses) is needed for consolidating memories, using a method that induces LTD would, in principle, disrupt memory (Muellbacher., 2002). Since rTMS can be

used to induce LTD, one can use rTMS in order to interfere with the motor memory consolidation process (Censor and Cohen, 2011).

In principle, measuring LTP after memory encoding would allow one to infer whether a memory is likely to be retained or not. Since LTP cannot be directly assessed in humans, researchers have designed alternative protocols to estimate LTP non-invasively. For example, one way to infer LTP-like mechanisms in cortico-spinal pathways is to assess CSE, which provides a broad measure of the excitation and inhibition status of the cortico-spinal system (Kleim et. al 2007). The primary motor cortex (M1) is an area of the brain engaged in multiple forms of motor skill learning (Hardwick et al., 2013), which is actively involved in the early consolidation of procedural memory (Muellbacher et al., 2002). When TMS is applied to M1, it elicits motor-evoked potentials (MEPs), a rough quantification of the level of CSE, from contralateral peripheral muscles (Robertson et. al, 2012; Rothwell et. al, 1987; Di Lazzaro et. al, 2004; Rothwell et. al, 1999; Chen et. al, 2008; Terao et. al, 1995; Barker et. al, 1985; Mills et. al, 1987; Day et. al, 1987). Quantifying the amplitude of the MEPs provides a broad estimate of the level of excitability in cortico-spinal pathways and other interconnected structures (Bestmann and Krakauer, 2015).

We have shown that chronological age is associated to weaker motor memories (Roig et al. 2014). Deficits in the consolidation of motor memory in older adults are commonly attributed to an impaired capacity to trigger neuroplastic mechanisms such as LTP during motor practice (Barnes, 2003). Advancing age has been associated with reduced capacity for LTP and LTD-like changes after exposure to rTMS protocol (Todd et al., 2010). However, there is ground to think that this lack of neuroplasticity (Todd et al., 2010; Freitas et al., 2011) may paradoxically shield older people from memory interference induced with NIBS during the consolidation phase. This is quite paradoxical as this would suggest that the lack of neuroplasticity hinders the elderly from achieving solid motor memory but also protects them

from memory interference when induced with NIBS methods that require synaptic plasticity to be manipulated. Therefore, this means that their 'weak' memory will not get disrupted through NIBS-induced interference as easily as with behaviorally-induced interference using ABA paradigms for example (Roig et al., 2014).

1.3 Objective

The objective of this study was to determine differences between young and older people in the response to a repetitive TMS protocol (rTMS), designed to induce LTD-like mechanisms and interfere with the motor memory consolidation process, in relation to: a) CSE assessed from M1 and b) motor memory consolidation. By understanding the differences between how young and old subjects' motor cortex react after receiving LTD-like signals, valuable insight into the underlying mechanisms of motor memory consolidation and how aging affects them may be gained. .

CHAPTER II: BACKGROUND

This section brings an overview of the different types of memory and the different stages through which a (motor) memory trace must go through to become a long-term memory. Second, I will summarize motor memory interference and its types. I will also describe some of the effects of healthy aging on the nervous system and how this process modifies neuroplasticity, and by extension, the ability to form new memories. This section will focus particularly on LTP and CSE, a non-invasive surrogate of LTP, that can be assessed with transcranial magnetic stimulation. Lastly, this section will address CSE and its association with motor memory within the context of interference and aging. As a physician and a student passionate about geriatrics, neuroscience and rehabilitation, my interest is to investigate and understand the mechanisms involved in the consolidation of motor memory; in doing so, the acquired knowledge may increase our ability to design effective rehabilitation strategies to improve memory in different clinical groups.

2.1 Aging, neuroplasticity and memory

Aging is associated with a decline of many cognitive and motor brain functions (Deary et al., 2009) and, consequently, with a decrease in social and functional independence (Spirdusso WW, 2005). Moreover, it is already known that aging is also associated with a reduction in the number of synapses and thus the potential for neuroplasticity (Adams, 1987a). However, the consequences of this lack of neuroplasticity in the capacity to form strong and durable memories are not yet well fully understood.

2.2 Types of memory

Memory is the ability that one has to recall an information acquired earlier. One of the most common classifications to understand the phenomenon is the one proposed by Squire and Zola in 1991, which divides memory into two subgroups: declarative and non-declarative memory. Briefly, declarative (or explicit) memory stores information about facts or events and

is consciously accessible. In contrast, non-declarative (or implicit) memory is non-conscious and involves the learning of actions, habits, and motor skills (Squire & Zola-Morgan, 1991). Non-declarative memory encompasses four memory subtypes: simple classical conditioning; non-associative learning; priming; and motor memory, also called procedural memory. New motor skills are acquired through motor learning, which is the process of improving the performance of motor skills through motor practice (Dayan and Cohen, 2011). The motor memory formation process is affected by the aging process (Seidler et al., 2010) and it has an important role in motor rehabilitation.

2.3 Stages of memory formation

The first stage to form a new motor memory is called encoding (i.e., online learning), which is the stage where sensorimotor information is acquired, and motor memories start to be formed by engaging or performing an action. Following the encoding stage, the next stage of motor memory formation is consolidation. During consolidation, the motor memory can be progressively strengthened or simply disappear (Brashers-Krug et al., 1996). Indeed, in this stage, an initial fragile memory can be transformed into a robust and stable memory or can simply end up in forgetting (McGaugh, 2000). If the memory is not forgotten, consolidation may lead to the stabilization of the memory trace but also to an enhancement (Robertson et al., 2004b). When a memory trace becomes stable, the same skill level can be observed at retention. In contrast, an enhancement of the memory trace leads to off-line improvements that occur even without additional motor practice (Robertson et al., 2004b).

It has been well documented that aging affects the encoding and consolidation of motor memories (Fleischman et al., 2004). There are numerous mechanisms that can explain the decline in neurobehavioral functionality in older adults. For example, neuronal morphology changes and the healthy aging are, inevitably, accompanied by cortical gray matter atrophy (Good et al., 2001). There are also changes in synaptic function in older adults (Adams, 1987;

Eisen et al., 1996), for example, a decrease in the connectivity between synapses in the motor cortex (Seidler et al., 2010) and in the concentration of neurotransmitters (Robinson, 1975; Zahr et al., 2008). However, compensatory mechanisms during the retrieval of motor memories have been reported in aged adults (Mattay et al., 2002), which are typically characterized by over activation of other brain regions (Cabeza et al., 2002). Although this "over activation" of the aging brain is well documented, the neural mechanisms causing this compensation and how it contributes to optimize the consolidation of the motor memory remain unclear.

The processes underlying the consolidation of the motor memory and how aging impacts them are not well described in the current literature. Therefore, understanding this stage of memory formation is fundamental for the development of interventions that can improve the capacity to form strong motor memories in the elderly. As mentioned before, during consolidation, the motor memory can become more robust and less susceptible to be disrupted. However, the performance of the skill learned before can also decrease when compared to the antecedent measure of the same skill. This can happen naturally or when there is a perturbation right after the encoding phase. This perturbation is known as memory interference, which is a useful experimental tool to investigate memory consolidation because it allows testing the strength of a memory during its formation (Robertson, 2012).

2.4 Motor memory interference

Motor memory interference has helped understand how memories are organized within the human brain. Recent studies have given new insights into the stabilization of a new motor memory, which makes it resistant to interference, guiding to a better understanding of its consolidation (Robertson 2004a). A new memory can be susceptible to interference right after its encoding. However, a series of neurophysiological changes during consolidation can make it resistant to inference several hours after the encoding (Dudai et al., 2004; Robertson et al.,

2004a). These mechanisms, that are responsible for making the memory strong and robust, can be disrupted creating, thus, interference.

There are different ways of creating interference during memory consolidation. In behavioral interference paradigms, the recently encoded memory is disrupted by learning a second motor task right after initial motor practice (Brashers-Krug et al., 1996). There is also pharmacological interference, whose objective is to block the synthesis of proteins related to memory consolidation using medications. Lastly, interference can also be caused by TMS, disrupting neural activity after encoding (Censor et al., 2011; Robertson et al., 2005; Muellbacher et al., 2002). Overall, the manipulation of the mechanisms responsible for the consolidation of memory have improved the knowledge about memory processing (Robertson et al., 2004a). However, a more detailed understanding is necessary. Moreover, it is necessary to deepen the understanding of how this process is modified with aging and its neurophysiological underpinnings, such as the reduction of neuroplasticity. Better understanding of these mechanisms would allow for the development of methods that improve the long-term retention of a memory and, thus, bring clinical benefits (Robertson, 2012).

2.5 Neuroplasticity and LTP

Neuroplasticity, or "brain plasticity", is the capacity of the brain to change, adapt, and create new connections in response to different stimuli. The human motor cortex is capable of reorganization (Todd et al., 2010) and, thus, create or modify the strength of new synapses. This is a continuous process and occurs as one learns and memorizes new information. Neuroplasticity is, thus, the biological underpinning of the learning brain (Taubert et al., 2010). Although the ability of the brain to learn and memorize new information is not fully understood, recent studies can provide us with important insights.

Plasticity is believed to arise from processes that involve strengthening, also called LTP, or weakening, also called LTD, of the synapses (Sanes et al., 2000). From experiments

using animal models it is already known that there is a relationship between the age-associated decline in synaptic plasticity and neurocognitive impairments (Barnes, 1979). In aged rodents, the thresholds for induction of LTP increase and LTD decrease in the hippocampus (Rosenzweig et al., 2003). Moreover, deficits in the capacity to maintain LTP after practicing a motor task in older rats have been associated with a greater degree of forgetfulness (Barnes and McNaughton, 1980; Kelly et al., 2006). Since LTP cannot be directly measured in humans, scientists have developed techniques such as transcranial magnetic stimulation (TMS) to assess it non-invasively (Hallett, 2007). TMS is particularly useful to explore mechanisms sub serving motor memory and skill learning processes (Censor and Cohen, 2011), including changes in CSE (Breton and Robertson, 2014).

When TMS is applied to M1, a magnetic field creates an electric depolarization of the cell membranes of motor neurons, eliciting MEPs, which become especially evident in contralateral peripheral muscles (Rossini et al., 2015). Quantifying the MEP amplitude provides an estimate of the excitability of cortico-spinal pathways and other interconnected structures such as spinal interneurons. Importantly, although CSE is usually regarded as a surrogate of LTP, it is important to emphasize that the MEP is a broad measure of synaptic plasticity (Bestmann and Krakauer, 2015), which can be influenced by the activity of several brain neurotransmitters (e.g. glutamate, GABA) that, in turn, can modulate the balance between the excitatory and inhibitory activity of neurons in the entire cortico-spinal axis (Ziemann et al., 2006b).

2.6 CSE and motor memory

Changes of CSE in the human brain are consistent with the properties of LTP/LTD (Baranyi and Feher, 1981; Hess and Donoghue, 1996; Castro-Alamancos et al., 1995; Hess et al., 1996; Hess and Donoghue, 1996). Thus, because of the similarity, these changes are called LTP/LTD-like plasticity. In the light of the new findings, recent studies have been providing

insights on how motor memory may be formed at the level of synaptic plasticity. In general, findings support that the behavior of LTP-like mechanisms contributes to motor learning (Ziemann et al., 2004) and, with the advance of age, the capacity to trigger LTP is hampered (Freitas et al., 2011; Todd et al., 2010), suggesting that age-related deficits in motor memory could be explained by the incapacity to trigger enough LTP.

In addition, recent studies have shown an association between CSE, assessed through the stimulation of M1, and the consolidation of the motor memory both in young (Tunovic et al., 2014; Ostadan et al., 2016) but also elderly people. All together, these studies suggest that changes in CSE are linked with the mechanisms that are responsible for the consolidation of the motor memory. More specifically, the results showed that a decrease in CSE after the encoding leads to no off-line improvements (Tunovic et al., 2014) and, in contrast, increases in CSE immediately after motor practice predicted improvements in motor memory (Ostadan et al., 2016).

Motor learning is essential for maintaining independence and health, recovering from injuries and, thus, reducing the expenses of health systems (Coats et al., 2014). However, older adults tend to exhibit more difficulties while trying to improve a motor skill or need more training in order to achieve the same skill level than younger counterparts (Coats et al., 2013; Smith et al., 2005). Moreover, the motor memory encoded by older adults appears to be less robust and more susceptible to interference (Roig et al., 2014). Motor memory interference can be used to achieve a better understanding of the mechanisms underlying the memory consolidation. In summary, it is already known that older adults are more susceptible to behavior interference (Roig et al., 2014). However, the effects of creating motor memory interference in older adults with TMS are not well fully understood.

CHAPTER III: METHODS

3.1 Study Design

This is a mixed design study with two subject groups: young and elderly healthy adults.

3.2 Study Subjects

The sample consisted of 29 subjects (15 young and 14 old). Subjects *were included if they were*: within the required age limits (young: 18-35; elderly: 55-85); to decrease variability among subjects, only right-handed subjects were tested; Moreover, they should be naïve to the motor task of the experiment. Only healthy subjects were tested, i.e. no neuropsychological disorders (absence of motor and sensory impairments), no arthritis or any type of hand injury, which could potentially affect one's performance on the serial reaction time task (SRTT) used in the study. *Exclusion criteria were:* any neuropsychological conditions (e.g. dementia, stroke, Parkinson's), the use of any recreational and/or medicinal drugs, which might affect the central nervous system, any contraindication to being exposed to TMS (e.g. depression, epilepsy, pacemaker user, self-reported history of high alcohol consumption - 5 or more alcoholic drinks for males or 4 or more alcoholic drinks for females on the same occasion on at least 1 day in the past month -, etc.) (National Institute on Alcohol abuse and Alcoholism) and deviates two standard deviations (SDs) from the age normative score on the NIH Toolbox Cognition Battery. Pianists or professional video gamers were also excluded from participation because the motor task used in this project involves sequential finger movements that they can be accustomed to. All subjects signed a consent form to participate in the study. Ethics approval for this study was received from the institutional Ethics Review Board.

3.3 Sample size estimation

The program G-power was used to estimate the sample size required to detect differences between groups in M1 excitability and skill retention. Using previous data, we calculated a conservative difference effect (f) between groups of 0.3 for motor memory (Roig et al., 2014) and CSE (Todd et al., 2010). With this effect size, 16 subjects per group were needed to detect differences at an alpha level of 0.05 and a beta power of 0.9. In this study, the sample size consists of 15 young subjects and 14 elderly subjects. We are still testing to complete our final sample size estimation. Moreover, in case of drop-outs or exclusions, new subjects will be recruited until reaching the desired sample size.

3.4 General procedures

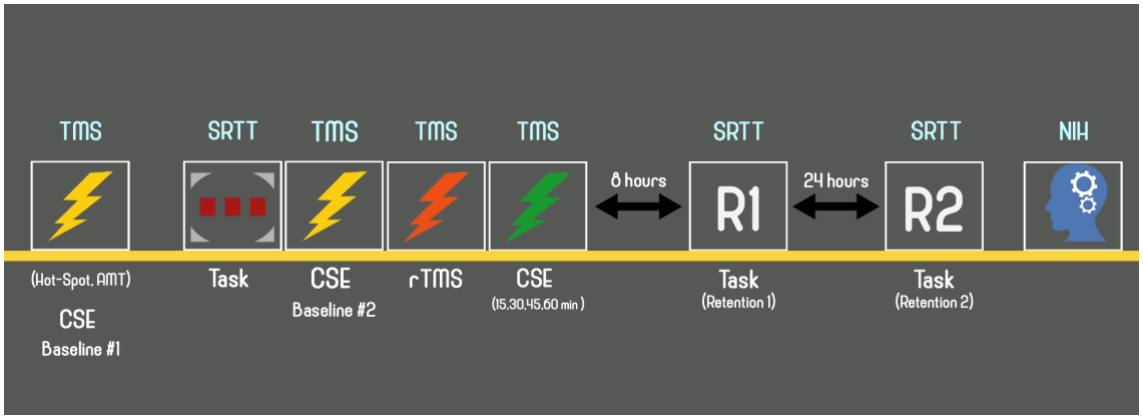

Fig. 1 *Phases of the study.*

Phases of the study occurred as outlined by **Fig. 1**. Subjects were asked to arrive at the laboratory at the Jewish Rehabilitation hospital by 9 a.m. Prior to the first CSE measure, the 'hot-spot' at the M1 region was located with a mini-mapping procedure and the resting motor threshold (RMT) was determined. Once the preparatory measurements were duly noted the first CSE measure was taken (baseline 1). The practice of the SRTT followed the baseline 1. A second measure of CSE was done after the task (baseline 2). rTMS was applied immediately after baseline 2. Previous studies suggest that the motor memory is more susceptible to the

effects of rTMS right after the encoding (Muellbacher et al., 2002). Subject's CSE level was assessed 15, 30, 45, 60 minutes after the task. M1 seems to be an important neural substrate in the early consolidation of motor memories encoded during the practice of this SRTT (Press et al., 2005). Moreover, two retention tests of the SRTT were performed. The first test was performed 8 hours after motor practice and the second twenty-four hours after. These two tests allowed to evaluate sleep-independent (8 hours) and dependent (24 hours) effects of rTMS on motor memory consolidation. Subjects were asked to avoid exercise, sleep and drink coffee during the eight hours before the first retention test. A questionnaire to collect this information was administered during the first retention test.

3.4.1 Transcranial Magnetic Stimulation Procedures

To apply the TMS protocol, the optimal stimulation region on M1 must be first found. This region, commonly referred as "hot-spot", provides an optimum MEP of the First Dorsal Interosseous (FDI). The cortical representational area of the FDI muscle was targeted, as it is deeply involved in the SRTT task and has a lower RMT (Rossini et al, 2015). The participant's head anatomical landmarks were recorded using a neuronavigation system and was co-registered to a standard magnetic resonance image template. In order to detect the "hot-spot" stimulation of the brain, the TMS coil was positioned at a 45° angle to the midsagittal line (to ensure optimal activation of cortico-motoneuronal cells). After the hot-spot was identified, the RMT was defined. The RMT can be explained as the minimum intensity needed to elicit a MEP of 0.05 mV in 10 out of 20 stimulations (Rossini et al., 2015). A low RMT is crucial as it implies that a lower absolute intensity stimulation is needed when using TMS to evoke a MEP. This fact is particularly relevant as it has been shown that elderly subjects usually require higher stimulation intensity to elicit a motor response (Freitas et al., 2011). Responses to the TMS were assessed via electromyography. Two AgCl surface electrodes were placed over the right first dorsal interosseous muscle. Signals were filtered using SENIAM recommendations.

The rTMS protocol consisted of stimulations given at 1 Hz (each pulse repeated every second for a total of 600 pulses (10 minutes of stimulation). The intensity of the rTMS protocol was set at 90% of the RMT (Robertson et al. 2005). For the CSE measures, a slightly modified version of Tunovic et al's protocol was used: 2 blocks of 20 pulses at 120% of the RMT at baseline (baseline 1), immediately after the SRTT (baseline 2) and at 15, 30 45 and 60 minutes after the end of the task were delivered (Tunovic et al., 2016). We have used a similar protocol in previous studies (Ostadan et al., 2016). This intensity (120% RMT) corresponds to the rising phase of the stimulus–response curve, where there is a roughly linear increase with TMS intensity (Rossini et al. 2015). To minimize the potential effects of repetitive TMS on CSE and procedural memory (Muellbacher, Ziemann et al. 2002), each stimulation was separated by 5s in between.

3.4.2 Serial Reaction Time Task (SRTT)

The SRTT has been extensively used to measure implicit learning (Robertson et al., 2007). This task was used because it has been shown to engage similar networks in young and older individuals (Daselaar et al, 2003). It requires pressing the correct keypad (out of a four-keypad option) after being presented with a stimulus shown on the computer screen. The entire sequence of the task is composed of twelve items (2-3-1-4-3-2-4-1-3-4-2-1). If the participant makes a mistake, the stimuli on the screen will remain until the correct key is pressed. However, if the answer is correct the cue on the screen disappear and another one appears 400 ms after. The signal consists of black blocks shown on the computer screen (a 23" monitor positioned at approximately 0.5m away from the subject's face); each block corresponds to a particular key option on the pad. The association between the computer blocks and the hand pad (**Fig. 2)** is based on location (i.e. the blocks shown on the computer are aligned from left to right: one left block, one middle-left block, one middle-right block, one right block and each of these blocks

is assigned to a keypad by the same logic i.e. left block with left key, middle-left block with middle-pad and so on).

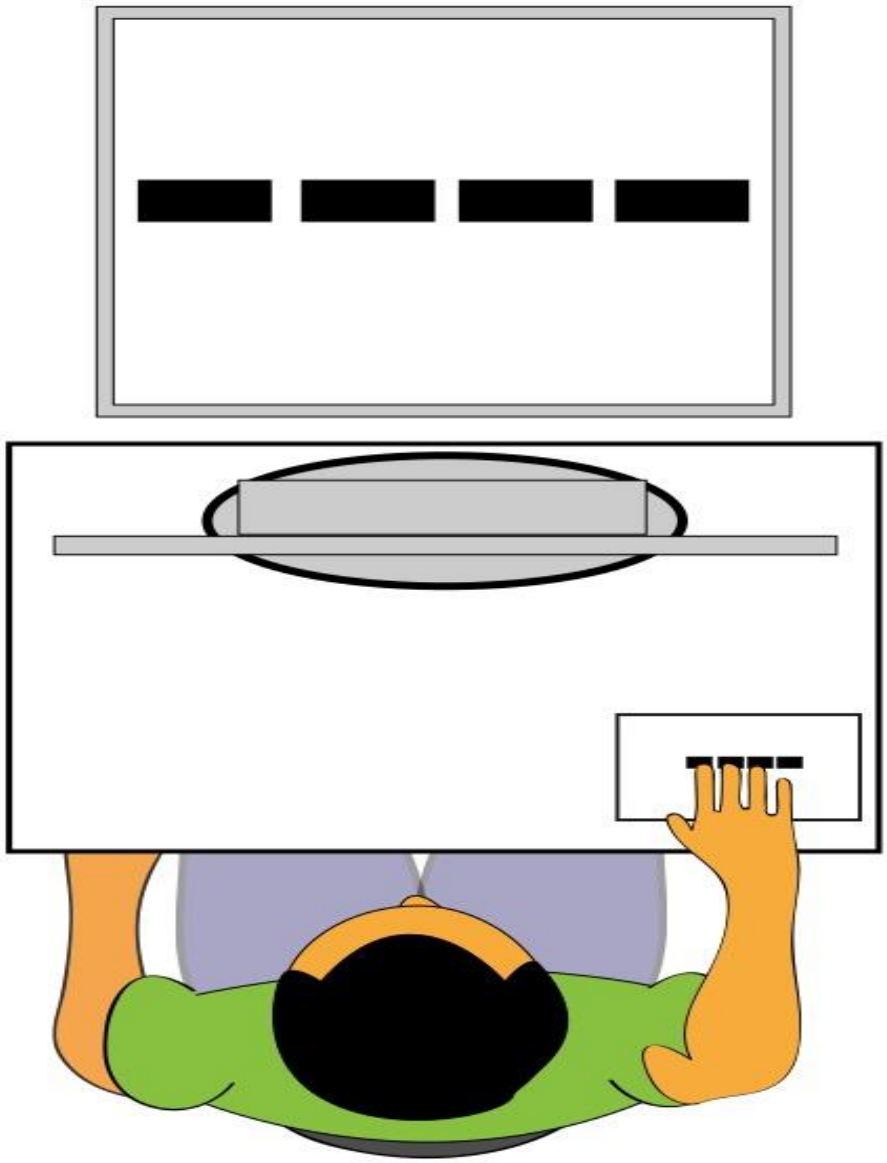

Fig 2. *Subject performing the SRTT.*

The SRTT task has been shown to be a reliable and valid task that allows measurement of motor memory in young and older individuals (Brown and Robertson. 2009). The first contact with task (encoding) consisted of three blocks, 15 repetitions of the 12-item sequence (total of 180 trials), followed by a main block of 25 repetitions (total of 300 trials) and then another block with 15 repetitions (**Fig. 3**). When subjects are tested and retested in the sequence

with small intervals of time does not increase their skill (Robertson et al., 2004b). The retentions tests consisted of one block of 5 repetitions of the sequence. Each time that the sequence was performed it was preceded and followed by 50 random trials (no specific item sequence) (Robertson et al., 2005). After the second retention, a free-recall test was applied to check the awareness of the subjects for the underlying sequence. Awareness was defined as more than four consecutives correct items of the sequence (Willingham and Goedert-Eschmann, 1999). All subjects with five correct answers or more were excluded.

3.4.3 NIH Toolbox

The NIH Toolbox is a set of tools used to assess cognitive, emotional, sensory and motor functions through computerized tests. Its use has been validated in a wide range of ages (3 to 85) across different ethnic, cultural groups. Subjects were seated in front of a monitor and asked to solve tasks involving different cognitive domains. The tests is administered in 25 minutes and provide specific and summary scores. In our study, we used this toolbox to identify deficits in both episodic and working memory (Episodic and Working memory Score). Subjects with values 2 SDs below the group mean were excluded from the study. A full description of the tests, normative values and scoring instructions is available at: http://www.nihtoolbox.org/Pages/default.aspx.

3.4.4 Sleep Questionnaire

Subjects completed a sleep questionnaire on their first and second visits. The questionnaire was used to identify subjects who had an abnormal sleep pattern by evaluating the self-reported quality and quantity of sleep on the previous night. The quality of sleep based on the information provided by the subject was used to identify sleep disturbances and their impact in the outcomes of the study because poor sleep could potentially affect cognitive abilities, which could in turn skew data.

3.5 Data Analysis

Demographic data: Age and NIH of all subjects were collected. Moreover, data from the sleep questionnaire were transformed into categorical values.

Cortico-spinal Excitability (CSE): All stimulation trials collected every 5 seconds were visually inspected before analysis. Frames with observable movement artifacts and excessive signal noise before the stimulation were tagged and removed from the analysis. The MEPs amplitude (peak-to-peak) was calculated for each frame and the CSE value is the mean of the all frames of the same time point.

TMS was used to investigate levels of CSE at baselines (1-before task- and 2-after task-) and during different time points (15, 30, 45 and 60 min) in the 1-hour period following motor practice. For each time-point 40 frames were collected. The baseline 1 was measured to check differences between age groups before the experiment. The other time-points were normalized to baseline 2 (CSE after motor practice), to factorize individual differences in CSE (Tunovic et al., 2014). Moreover, the difference between the time point "15 min" and baseline 2 was calculated to evaluate the immediate effects of the rTMS (i.e. rTMS effect).

Global changes in CSE during consolidation for each participant were estimated by calculating the area under the curve (AUC). Briefly, with the normalized CSE measurements of each subject obtained at different time points (baseline 2, 15, 30, 45 and 60 min) it was possible to calculate the AUC in Matlab® using the trapezoidal function. This function allows the determination of global changes in excitability along different time intervals accurately (Ostadan et al., 2016).

Motor skill encoding and retention: Skill performance in the SRTT was quantified, using a custom application built on Superlab as the time to respond to each visual cue. Performance was calculated from the last block of each session by subtracting the average

response time of the final 50 sequential trials from the average response time of the subsequent 50 random trials (**Fig. 3**). Using the difference between sequential and random trials removes the potential influence of fatigue and other factors (variations in movement speed) that could mask changes in motor sequence learning (Nissen and Bullemer, 1987; Willingham et al., 1989; Willingham and Goedert-eschemann, 1999). Only trials with correct responses were considered for the analysis and response times that deviated 2.7 SDs or more from the mean were removed (Robertson et al., 2005). Using this procedure, skill level of the test block of motor skill encoding (Skill 1) and the retention blocks (Skill 2 and Skill 3) were calculated (**Fig. 3**). The difference (delta value=Δ) between skill level during motor learning and retention (Skill 2 - Skill 1) provides a measure of off-line improvements during consolidation (non-sleep dependent - Δ1 -), while Δ2 (Skill 3 – Skill 2) and Δ3 (Skill 3 – Skill 1) provides a measure of sleep-dependent consolidation.

Accuracy was not considered as a measure of skill performance because the number of errors in this motor task has been proved to be very low (Robertson et al., 2005). However, the number of errors in both sequential and random blocks was quantified to determine if potential deviations in speed-accuracy trade-off might influence the time to respond in any of the groups. Moreover, a comparison between the response time of the last 50 sequential trials of each session (Test –T-, retention 1 –R1- and retention 2 –R2-) was also performed to check the possibility of changes between test and retention. Deltas (Δ) between the response time of the sequential blocks of each session were also calculated (ΔSeq T – R1, ΔSeq T – R2 and ΔSeq R1 – R2). It is known that older adults have similar results in comparison to young adults in sequence-specific learning (Howard et al., 1989; Howard et al., 1992). Thus, to check if the subjects learned only the sequential part of the task, the same analysis was also done for the random trials and the deltas were calculated to ensure that there were no improvements between

sessions.

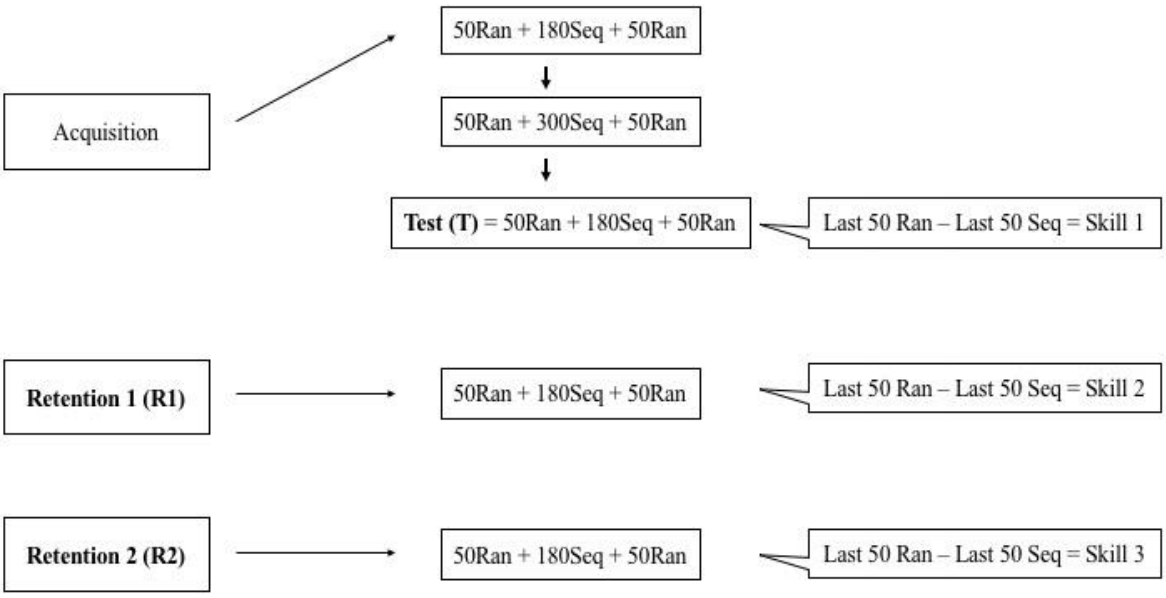

Fig. 3 *Scheme of the SRTT's analysis.*

3.6 Statistical Analysis

All statistical analyses were performed using IBM® SPSS® version 24. The *Shapiro-Wilk test* was used to examine the distribution of the data for all variables within each group. Differences between groups young and elderly in memory scores (WMS and EMS), age, hours of sleep during the night and RMT were assessed with *independent-samples t test*. Differences in the morning feeling were assessed with the *Chi-Square test*. To take into consideration differences between sleep dependent and non-sleep dependent consolidation of the motor memory, skill 1, 2 and 3 were analyzed separately. Thus, between groups differences in motor skill encoding, retention 8h and 24h as well as differences between sessions in the response time of the sequence and random trials were assessed with *independent-samples t test.* Differences between groups in CSE baseline 1 and 2 were assessed with *independent-samples t test*. The effect of the task between the two baselines was also assessed with *paired-samples t test.* Changes in normalized CSE across the different time points (15, 30, 45 and 60 min) were

examined with *two-way (group x time) repeated measures ANOVA*. When the *ANOVA* violated the assumption of equal sphericity, the *Greenhouse-Geisser* rectification method was applied. We used the AUC to determine whether global changes in CSE are associated with procedural memory. Correlations between AUC, motor skill encoding and retention (8h and 24h) and differences in the sequence time of each session were explored independently for each group with *Pearson's or Spearman's correlations* depending on the distribution. Unless otherwise reported, data are presented as means and standard error of the mean *(SEM)* and all analyses performed with two-tailed probability test with the statistical level set at $p<0.05$.

CHAPTER IV: RESULTS

One participant (1 young) with very high threshold, who required extremely high TMS intensities, did not go through the CSE assessment. However, this participant received rTMS and her behavioral data were included in the analyses assessing skill encoding and retention. Moreover, the data of one participant in the young group, who had an unusual negative skill 1 (the value was more than 3 SDs smaller than the group mean), were excluded.

The RMT was higher in the young group (young=59.35 [2.36]; old=57.92 [1.65]) but differences between groups did not reach statistical significance (*independent-samples t test*; t=-0.494; p=0.625). **Table 1** shows the demographic characteristics of each age group, including the results of the sleep questionnaire. Age, memory scores and morning feeling were significantly different between groups.

	Young	**Old**
Subjects (n)	14	14
Age (years)*	27.79 (5.01)	70.64 (7.65)
Sex (M/F)	5/9	5/9
Memory		
*Working memory (score)**	101.64 (12.32)	106.72 (18.81)
*Episodic memory (score)**	117.71 (12.91)	99.56 (13.63)
Sleep		
Total sleep (hours)	7.17 (1.69)	7.64 (0.81)
Wake up at night (times)	0.64 (0.92)	1.14 (0.94)
Morning feeling*		
Refreshed (n)	11	4
Somewhat refreshed (n)	3	6
Fatigued (n)	0	4

Table 1. *Demographic characteristics of the subjects of the study. Data are provided as means and standard deviation of the mean (SD). * ($p \leq 0.05$).*

4.1 Effects of the interference on skill retentions

There were no statistically differences between skills (Skill 1 = *independent-samples t test: t = 0.708, p = 0.485;* Skill 2 = *independent-samples t test: t = 0.805, p = 0.428;* Skill 3 = *independent-samples t test: t = -0.401, p = 0.692)* (**Fig. 4**). However, the relative improvement between skill acquisition during motor practice (Skill 1) and the first retention (Skill 2) was

greater in older subjects. In fact, young subjects improved skill level 30.69% and older subjects 38.61%. When the second retention (Skill 2) and the third retention (Skill 3) were considered, both groups showed a decay in skill level. However, in the old group, the relative decay was greater (53.71%) than in the young group (34.87%). For this reason, Δ1 (Skill 2 – Skill 1) was greater in the elderly subjects, but Δ2 (Skill 3 – Skill 2) and Δ3 (Skill 3 – Skill 1) are smaller (**Fig. 5A)**, showing a non-statistically greater improvement in non-sleep dependent memory consolidation in the elderly group and a more pronounced decay in the sleep dependent memory consolidation in the same group (Δ1 = *independent-samples t test: t = 0.458, p = 0.651;* Δ2 = *independent-samples t test: t = -0.982, p = 0.335;* Δ3 = *independent-samples t test: t = -0.869, p = 0.393).*

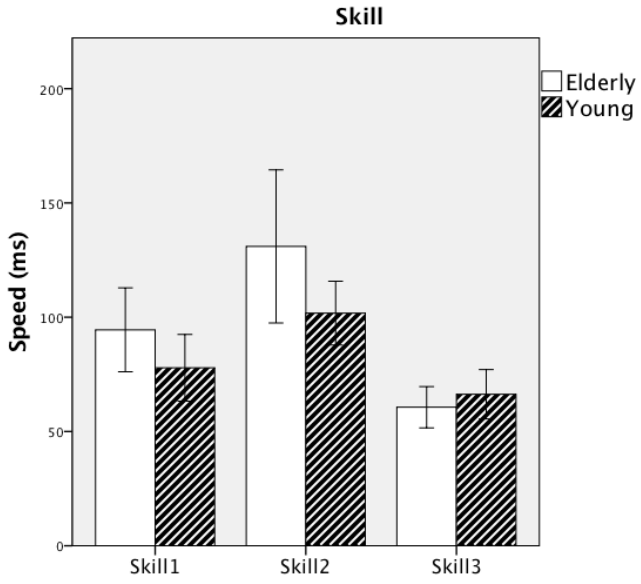

Fig. 4 *Comparison between skills. Errors bars are the Standard Errors (SE) of the mean.*

There were no statistically differences between groups in the deltas of the random part (ΔRan T – R1 = *independent-samples t test: t = 0.115, p = 0.909;* ΔRan T – R2 = *independent-samples t test: t = 1.255, p = 0.221;* ΔRan R1 – R2 = *independent-samples t test: t = 1.161, p = 0.256,* meaning that subjects did not encode the random part of the task. The difference

between sequential time at the encoding and the first retention (Δ Seq T – R1) and the encoding and second retention (Δ Seq T – R2) was higher in the old group (**Fig. 5B**), meaning that the old subjects were less susceptible to interference in the response time of the sequence during sleep dependent and non-sleep dependent consolidation. However, the difference between groups was not statistically significant (Δ Seq T – R1 = *independent-samples t test: t = 0.681 p = 0.502*; Δ Seq T – R2 = *independent-samples t test: t = 1.173 p = 0.251*). Considering this, when the random part of the task is not considered, elderly subjects have a better retention of the memory, sleep and non-sleep dependent.

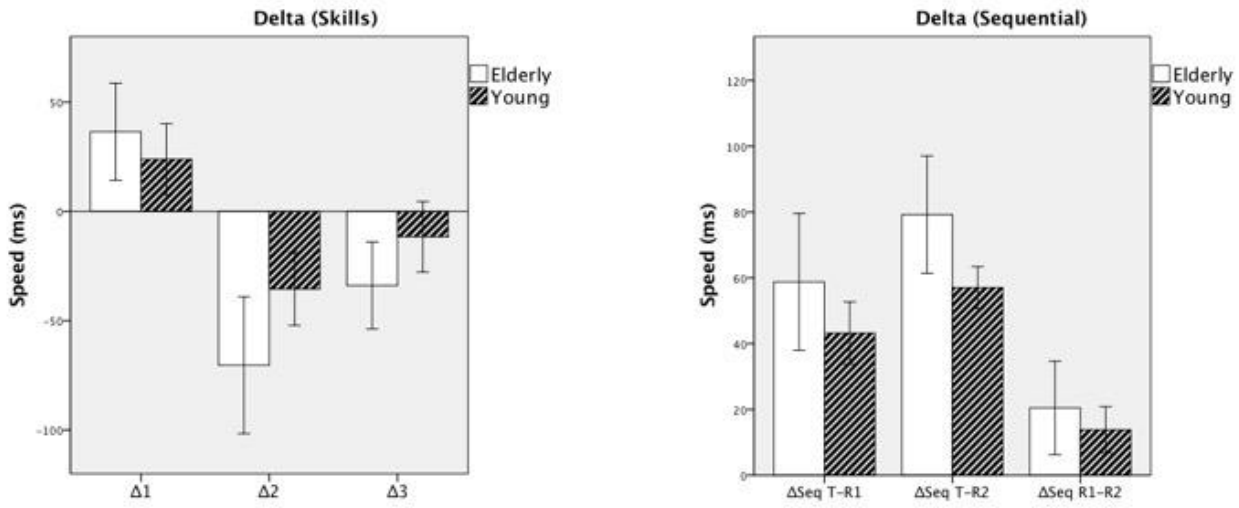

Fig. 5 A) *Deltas (Skills).* ***B)*** *Differences between the response time of the sequential part. Errors bars are the SE of the mean.*

4.2 Effects of the interference on CSE during consolidation

There was no difference between groups in CSE at baseline 1 *(independent-samples t test: t = -1.607, p=0.210)* or baseline 2 *(independent-samples t test: t =-1.009, p=0.323).* Moreover, the changes in CSE caused by the rTMS (applied between baseline 2 and the time-

point 15 min) were not statistically significant in any of the groups (Elderly = *paired-samples t test: t = 0.32, p=0.975; Young = paired-samples t test: t=1.3232, p=0.210).* To factorize differences in CSE after motor practice, each CSE time-point was normalized to baseline 2 (**Fig. 6)**.

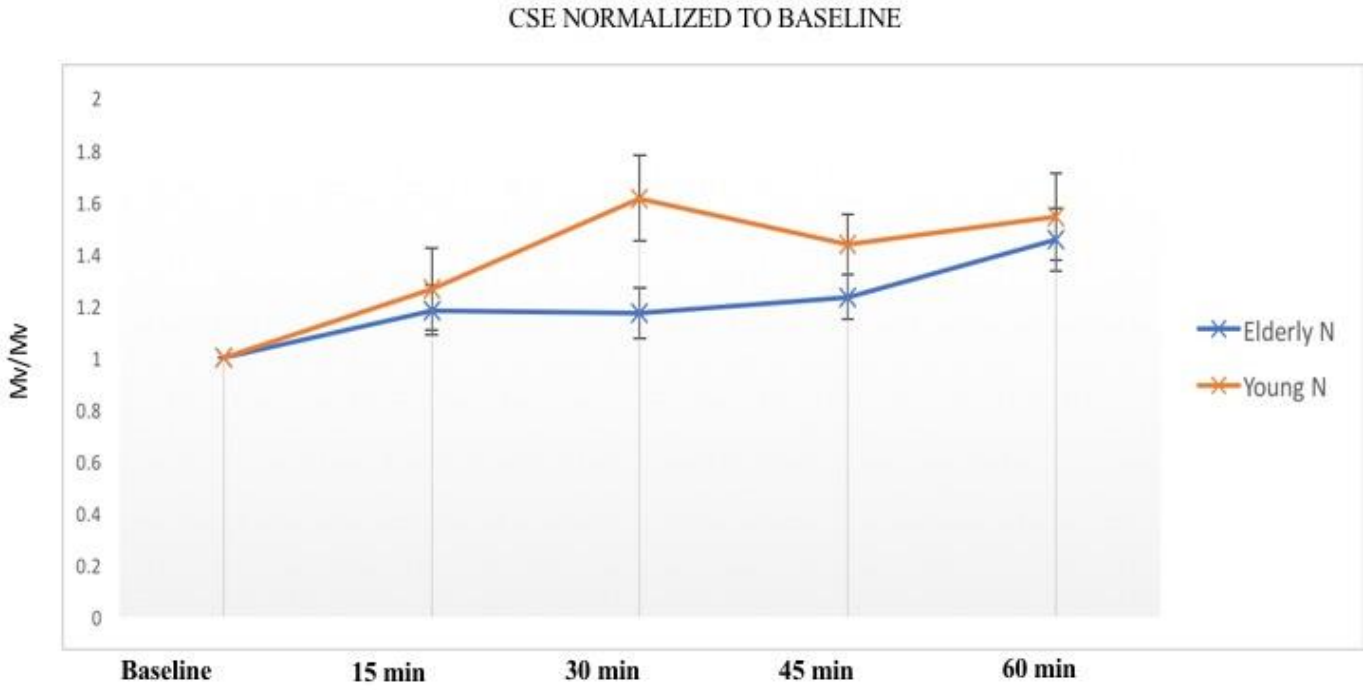

Fig. 6 *CSE normalized to baseline 2. Errors bars are SE of the mean.*

When compared to young, older subjects achieved the same level of CSE 60 min after practicing (encoding) the task (**Fig. 6**), with smalls increases at each time-point and a bigger increase after 45 min. The young group, however, presented a continuous increase until the time-point 30 min, with a decay afterwards. Nevertheless, there was no statistically difference between groups *(ANOVA, $F_{[2.49,67.44]} = 0.819$; $p = 0.477$)* in the different points. The corresponding AUC calculated from each line plot was smaller in the older group (**Fig. 7**). The difference was not significant between groups (*independent-samples t test: t = -0.606, p = 0.550)*.

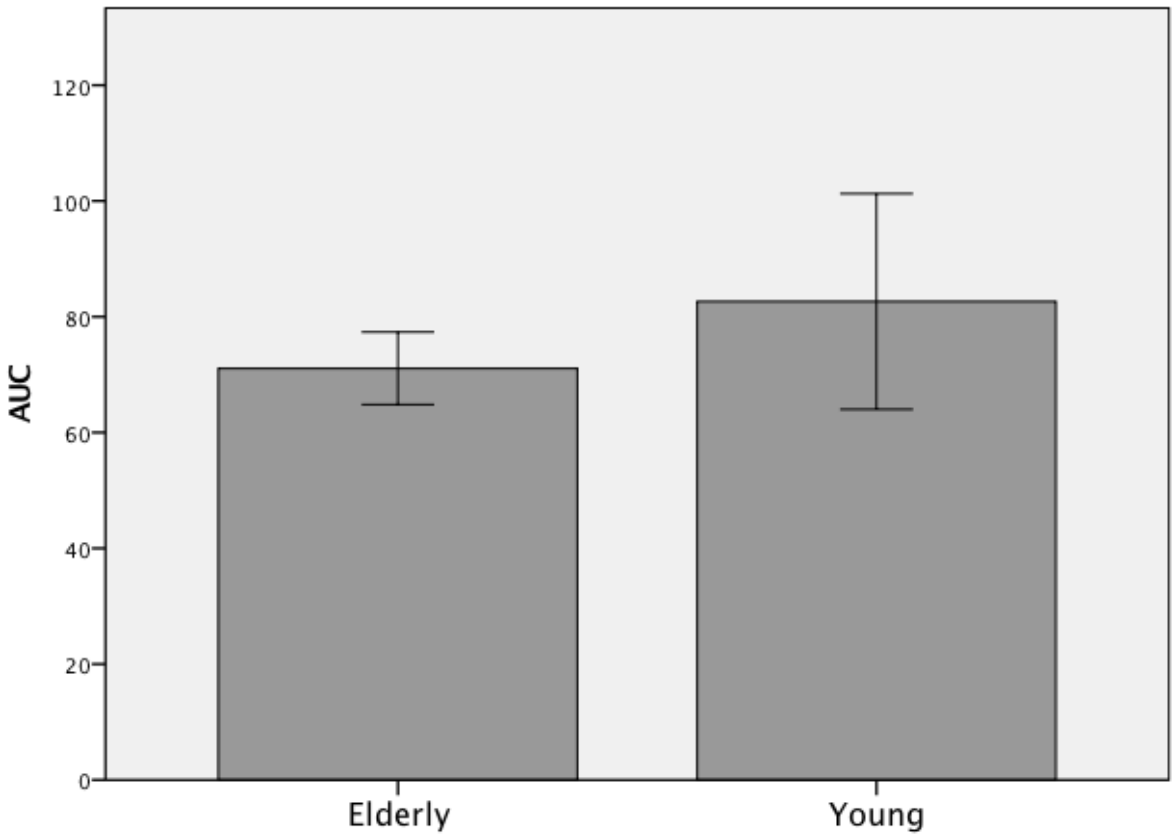

Fig. 7 *Area under the curve (AUC), an unitless measure. Errors bars are the SE of the mean.*

4.3 Association between CSE and motor skill retention

Correlations between absolute CSE and skill retention in old subjects revealed that older subjects who were less susceptible to the immediate effects of the rTMS (had a smaller decay in the CSE) improved the time in the response time of the sequence during the non-sleep dependent consolidation (*Spearman's correlation*; r = -0.574; p = 0.03). However, the effects of the rTMS were strongly related with global changes in CSE (*Spearman's correlation*; r = 0.76; p = 0.001). That is why, when normalized to baseline, subjects who increased more the CSE, improved more the response time of the sequence of retention 2 in comparison with retention 1 (*Spearman's correlation*; r = 0.6; p = 0.01).

In young subjects, the absolute value of the CSE showed a trend for significance when related to Δ3 (Skill 3 – Skill 1) (*Spearman's correlation*; r = 0.50; p = 0.078). However, the effects of the rTMS was also related to the changes in CSE (*Spearman's correlation*; r = 0.77; p = 0.002).

CHAPTER V: DISCUSSION

5.1 Effects of interference on skill retentions

Previous studies have shown that older adults have similar improvements when compared to young adults during the practice (i.e. encoding) of a motor sequence. However, during consolidation, contrary to what was observed in young subjects, older adults stabilized their knowledge while young had improvements (Brown et al., 2009). Therefore, a more profound knowledge about the mechanisms underlying the consolidation of the motor memory is necessary to better understand the aging processes of the brain.

Motor memory interference can be used to study the impacts of aging during consolidation because it tests the strength of the newly acquired motor skill (Roig et al., 2014). A decline in the performance of the motor task after interference is indicative of an incomplete consolidation. In contrast, the absence of decay is indicative of a successful consolidation (Brashers-Krug et al., 1996). It is already known that elderly subjects are more susceptible to behavioral interference (Roig et al., 2014). However, the neural substrates of this interference are not well understood. TMS is a NIBS technique that can be used to explore this.

When rTMS is applied to M1, areas that extend beyond M1 are also disrupted and these areas also have an important contribution in the development of improvements during memory consolidation (Robertson et al., 2005). However, there is a decrease of motor cortical neuroplasticity with age (Freitas et al., 2011) and, thus, the connection of M1 with the other areas might be altered. Therefore, we hypothesized that, paradoxically, older adults would be less susceptible to TMS interference in comparison to younger counterparts.

There were no statistically significant differences in skill level between groups. However, between the encoding and the first retention (8h after) older subjects showed a tendency for a better relative improvement. This result, together with previous findings showing deficits in the retention of the SRTT in older adults (Brown et al. 2009), suggest that

young subjects were more susceptible to TMS-induced interference. Previous studies (Brown et al., 2009) showed that while young subjects had improvements in between-sessions older subjects did not show improvements at retention. Taking this into consideration, we can conclude that in our experiment subjects had similar results because younger subjects were more susceptible to the interference, decreasing their skills, while older subjects were not less susceptible but still had deficits in memory consolidation.

It should be emphasized, however, that Brown et al. 2009 had only one retention 24h after motor practice. In our study, we had two retentions tests, 8 and 24h after. Thus, when only the sleep-dependent memory is considered, older adults had a more intense relative decay in comparison to young subjects. However, studies have been shown that a full night of sleep can mitigate the interference effects of rTMS (Robertson et al., 2005). Consistent with this, imaging studies show that during sleep other circuits that do not include M1 are responsible for the consolidation processes (Maquet et al., 2000; Maquet et al., 2003). Thus, for the sleep dependent memory, the susceptibility to the interference was reduced, apparently, in both groups.

5.2 Effects of interference on CSE during consolidation

Cortical excitability (CSE) provides a broad measure of functional changes in the motor circuits and it has been frequently used to identify the changes that occur after learning a new motor task (Sauseng et al., 2009; Yuan et al., 2011; Veniero et al., 2011). It is thought that the levels of CSE right after learning a new motor task signal memory consolidation processes, which explain the subsequent improvements during wakefulness (Breton and Robertson, 2014). Decays in CSE have been associated with no off-line improvements (Breton and Robertson, 2014). When a rTMS protocol (10 min at 1Hz), that reduces CSE, is applied to M1 off-line improvements are not achieved during wakefulness (Tunovic et al., 2014).

Nevertheless, recent findings suggest that M1 is not the only area of the brain responsible for memory consolidation. A broader circuit that goes beyond M1 might regulate this process. Thus, the development of off-line improvements is dependent on the ability of M1 to remain functionally connected with other areas of the brain that are involved in the motor memory processing (Breton and Robertson, 2014). It is already known, however, that this brain plasticity in the corticomotor area is reduced with aging (Freitas et al., 2011) and the connections of M1 with the motor circuit may be disrupted with the aging process. Therefore, since that the connection of M1 and the other areas of the motor circuit has an important role in consolidation mechanisms, we hypothesized that elderly subjects would be, paradoxically, less susceptible to the effects of the TMS interference, based on the lack of brain plasticity.

CSE was measured at different time-points for one hour after learning a motor task. Measuring CSE on a single point is not optimal because, even on a relatively short timescale, CSE can change substantially (Ostadan et al., 2016; Tunovic et al., 2014). More importantly, assessing CSE at one single point provides very little information regarding CSE levels during motor memory consolidation. To circumvent this limitation, we applied TMS at different points (Ostadan et al., 2016; Tunovic et al., 2014) and estimated the AUC in order to quantify absolute CSE level, but also the capacity to increase CSE in response to motor practice. Our results show no statistically differences between groups at the different time-points or in the AUC.

When normalized to baseline, the values of CSE of the older subjects were smaller than the young group. However, the older group shows small increases at each time-point while the young subjects present an important decay after the time-point 30 min. In the last time-point, both groups achieved a similar CSE level. Different results have been reported about CSE measures in old subjects. Previous studies show reductions in the capacity to increase CSE after motor practice (Rogash et al., 2009). However, other TMS investigations have not been able to demonstrate significant differences between age groups in CSE immediately after the

practice of a motor skill (Cirillo et al., 2010; Cirillo et al., 2011; Berghuis et al., 2016). Given these variable results reported in older subjects and that younger subjects show decays in CSE after repetitive TMS protocols designed to suppress CSE (Tunovic et al., 2014) we can conclude that young subjects had a decay in CSE and elderly subjects were less susceptible to the TMS interference.

5.3 Association between CSE and motor skill retention.

Correlations performed for each group, independently, showed that these associations followed different trends depending on the age group. We found that old subjects that were less susceptible to the immediate effects of the rTMS were the ones who improved more the response time of the sequence during the non-sleep dependent consolidation. This result is consistent with the hypothesis that older people would be less susceptible to the effects of rTMS and with previous findings showing that improvements in a motor skill level during consolidation can be sleep independent thus occurring during wakefulness (Spencer et al., 2006; Robertson et al., 2004b).

To understand the effects of the rTMS on the CSE, all time-points must be considered (AUC) because, as already mentioned, only one single point does not provide enough information about changes in CSE during consolidation. In this way, older subjects who were capable of increasing CSE more were also the ones who improved more the response time of the sequence of the second retention in comparison with the first retention. This result is the opposite of previous findings (Brown et al., 2009). In this study, the young group showed improvement in the 24h retention while older subjects maintained their level of skill. However, in our study there were two retention tests (8h and 24h) and interference with TMS immediately after the task. Thus, performing the first retention test might have strengthened the memory trace, diluting the effects of RTMs in the older group.

Moreover, it should be noted that between the two retention tests we had a night of sleep. It is already known that sleep has a really important role in the consolidation of the motor memory (Walker et al., 2002). While during wakefulness the brain selects what is going to be consolidated, during sleep declarative and motor skills can be consolidated simultaneously (Brown et al., 2007). The reason why this happens and the mechanisms that control the memory consolidation during sleep remain unknown (Brown et al., 2009). However, it is known that the rTMS was not able to disrupt overnight improvements (Robertson et al., 2005) possibly because memory improvements during sleep are dependent on circuits that do not involve only M1 (Robertson et al., 2005).

Nevertheless, our results are the opposite. While Robertson et al. 2005 tested only younger subjects, we had different age groups and the correlation between the increase in CSE and the improvement in the sequential time was observed only in older adults. The ability to keep brain plasticity and recruit other areas of the brain, besides of M1, is the key to explain why we had this result. It is known that the brain and the motor cortex, in particular, have a decay in neuroplasticity with aging (Freitas et al., 2011). However, compensatory mechanisms have been reported based on neuro-imaging while subjects perform motor tasks (Resnick et al., 2007). These mechanisms consist on over-recruitment of other brain areas (Cabeza et al., 2002). Paradoxically, the older subjects that had a lack of plasticity in M1 were capable of creating new connections with other brain areas during consolidation were the ones that had better results.

CHAPTER VI: LIMITATIONS AND CONCLUSION

6.1 Limitations

The main limitation of this study is the small sample size and the lack of control groups to determine whether changes in CSE and skill retention are due to rTMS or simply age differences. However, the results reported here correspond only to the first phase of the study. A control group is already being tested for future analysis. Regarding the motor task, the SRTT is a well-established motor learning paradigm to study procedural memory consolidation using NIBS. Nevertheless, we do not know yet to what extent the findings can be applied to more complex movements. Lastly, although the sleep criterion was assessed, there are various other factors that could potentially affect memory and cognitive performance that are not being assessed (i.e. psychological state like mental stress level, level of arousal like happiness/excitement due to extraneous events in the participant's life; physiological state like hunger, etc. is also not assessed).

6.2 Conclusion

Our results demonstrate that older subjects who were able to recover from the effects of the rTMS were the ones that improved more the response time of the sequence between the first and the second retention. The focus of our study was the motor cortex. However, we believe that older subjects were not susceptible to motor memory interference because M1 is just a part of the mechanisms responsible for the motor memory consolidation. Understanding the age-related changes in brain plasticity experienced by older adults, and how these changes can influence memory consolidation, provides valuable information regarding potential mechanisms subserving memory formation processes. Furthermore, understanding the neural mechanisms underpinning motor memory consolidation may increase our capacity to design novel approaches (e.g. exercise, non-invasive brain stimulation) to optimize memory in elderly

populations as well as patients with mobility impairments due to brain injury or neurodegeneration.

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Appendices

Subject Form

The Neuroplasticity Paradox: Can Lack of Neuroplasticity Protect Older Adults From Motor Memory Interference?

Date: _____
Name: _____
Phone number: _____
Group: _____

Subject
Age: _____
Weight: _____
Height: _____
Educational Level: _____
Impairments: _____

Inclusion Criteria
Healthy subjects 18-35 years old or 65-85 years old, right – handed, naive to the motor task.
Exclusion Criteria
Any clinical condition, medication, recreational drug that could affect nervous system, pianists, video gamers.

NOTE: Remind the participant of the following:
NO exercise during testing day
Follow normal daily routine but not take any stimulant (i.e. Coffee)
Avoid any type of arousal

DAY 1
Date: _____
Time: _____

Check the systems
- Superlab/ Labview
  - Task test
  - Force
- Calibrate Brainsight
  - Verify Polaris
  - Verify TMS coil
- Familiarize subject to TMS

Position of the subject
- LabView - Force test (MVC) – 2 timed w/ 30 s interval
  Score: _____ 20% of: _____
- Placement of hand
  Use sandpaper on FDI area
  Electrodes on FDI +ulnar head
  Proximal (+), Distal (-)
- CHECK THE SIGNAL
  - Make sure that the signal is not noise
  - Fix the electrodes

Finding the Hot Spot (Threshold file)
Hot Spot target = _____
Intensity of hotspot = _____

Threshold protocol (rest)

Check amplification (300/AC Couple/ 60 HZ notch) cutoff 500 Hz
Intensity at threshold = _____
120% threshold = _____
90% threshold = _____

Excitability protocol

- All excitability protocols
  - **(2x20 stimulations at resting with 2 min of resting in between them)**
  - Check amplification (300/AC Couple/ 60 HZ notch) cutoff 500 Hz
    Hand placement

- CHECK THE SIGNAL
  - Make sure that the signal is not noise
  - Fix the electrodes
- Baseline – Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

Task Protocol

- Serial Reaction Time Task (ACQUISITION)

☐ Hand positioning using pictures.
☐ Subject seated comfortably. Tape around response-pad.
☐ Measure distance of chair to table: cm _____
Computer:
Script: SRTT-INTCSE
SELECT: Instructions1, Initial Training, Break 1, Random Trials 1, Training, Random Trials2, Break2 Random Trials 3, Test, Random Trials 4, GoodbyeLearning.
« RUN SELECTED BLOCKS ONLY »
SessionID: _____
File Name: NP-_____
Start Time = _____
During breaks, say "Is everything okay?", "Remember to respond as fast and accurate as possible"
END TIME = _____
*Note: Reminder for participants

1. NO exercise/NO sleeping during testing day
2. Follow normal daily routine but take no stimulant drugs (i.e. Coffee OR alcohol)

Excitability protocol

- CHECK THE SIGNAL
  - Make sure that the signal is not noise
  - Fix the electrodes
- Immediately after finishing the task - Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

cTBS protocol – Immediately after excitability protocol

Excitability protocol

- Begin the protocol 15 after finishing the task. Then 30, 45 and 60 minutes after.

- 1 – 15 minutes - Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

- 2 – 30 minutes - Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

- 3 – 45 minutes - Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

- 4 – 60 minutes - Time: _____
  File's name: Excitability 00__

Comments: _____
_____
Total # of frames:

Retention test 1

- 8 hours after finishing the task - Time: _____
- Serial Reaction Time Task (RETENTION 1)

☐ Hand positioning using pictures.
☐ Subject seated comfortably. Tape around response-pad.
☐ Adjust chair to table: cm _____
SELECT: Instructions (Retention 8h), Random Trials 5, Retention 8h, Random Trials 6, Goodbye8h.
« RUN SELECTED BLOCKS ONLY »
SessionID: _____
File Name: NP-_____
Start Time = _____
During breaks, say "everything is OK?", "Remember to respond as fast and accurate as possible"
☐ File saved

Sleep Questionnaire

- Doyon
  - Mornings - 1st time
  - Evenings - 1st time

DAY 2

Retention test 2

- **24 hours after finishing the task - Time: _____**
- Serial Reaction Time Task (RETENTION 2)

☐ Hand positioning using pictures
☐ Subject seated comfortably. Tape around response-pad.
☐ Adjust chair to table: cm _____
SELECT: Instructions (Retention 24h), Random Trials 7, Retention24h, Random Trials 8, Questions strategy, Replication Instruction, Replication, Goodbye24h.
« RUN SELECTED BLOCKS ONLY »
SessionID: _____
File Name: NP-_____
Start Time = _____

During breaks, say “everything is OK?”, “Remember to respond as fast and accurate as possible”
☐ File saved

Questionnaire POST Serial Reaction Time Task (RETENTION 2)
“Did you use a particular strategy to perform the task?”
☐ YES ☐ NO , EXPLAIN: _____
“Did that strategy change during the task?”
☐ YES ☐ NO , EXPLAIN: _____

“How would you best describe the responses given during the experiment?”
☐ Random
☐ The succession of responses was often predictable
If yes, when: _____
☐ The same sequence was repeated throughout the task
If yes, when: _____

NIH
- NIH Test Login: _____
  Password: _____
- Working Memory Score: _____
  Sequence Memory Score: _____
  Handedness Test Score: _____

Sleep Questionnaire
- Doyon
  - Mornings - 2st time
- Pittsburg

MORNING QUESTIONNAIRE

Night of *a* _ _ _ _ / *m*_ _ / *j*_ _ to *a* _ _ _ _ / *m* _ _ / *j*_ _

1. What time have you got to bed (lights closed)? _____
2. How much time did it take you to sleep? _____ minutes
3. Have you woke up during the night? [_] No. [_] Yes. If yes, why?

Reason	**Number of time**	**Reason**	**Number of time**
Go to the bathroom		Hot flush	
Awakened by children or partner		Stress, anxiety, intrusive thoughts	
Noises-Hot-Cold		Simply woke up	
Physical discomfort (Cough, pain, etc.)		Other reason :	

- At what time? _____
- At what time have you got up?_____ Why had you to get up?:

	Alarm clock or someone needed to be awaken		Children
	Simply woke up		Hot flush
	Physical discomfort		Noises-Hot-Cold
	Stress, anxiety, intrusive thoughts		

4. How much time have you slept (excluding the awaken time and the laps of time to get to sleep)? _____ hours and _____ minutes.
5. To be in shape, do you feel that you slept :
   - [_] Long enough [_] Too much
   - [_] Not enough: Reasons [_] Obligation [_] Noise(s), Pain, discomfort(s) [_] couldn't sleep more
6. On a scale of – to +, draw a line where it corresponds best:

<u>The quality of the sleep:</u>

\- _____ +

Very bad sleep Very good sleep

<u>Your mood when you woke up:</u>

\- _____ +

Very tense Very calm

<u>Your level of alertness when you woke up:</u>

\- _____ +

Very tired Top shape

7. Do you remember having a dream? [_] No [_] Yes.
8. Do you remember having a bad dream (nightmare)? [_] No [_] Yes.
9. That dream woke you up? [_] No [_] Yes.

EVENING QUESTIONNAIRE

Night of a_ _ _ _ / m _ _ / j _ _

1. On a scale of – to +, draw a line where it corresponds best:

Your mood:

- _____ +

Very tense Very calm

Your level of alertness during the day:

- _____ +

Very sleepy Very vigilant

Your level of activity through the day:

- _____ +

Not much active Very active

Your form :

- _____ +

Very tense Very relaxed

2. Have you practice a violent sport or did a very intense workout today?
   - [_] No [_] Yes. If yes, which one? _____
   - At what time? _____
   - For how long? (minutes) _____
3. Did you take a nap today? [_] No [_] Yes. If yes, from what time? _____ to _____
   - Did you sleep ? [_] No [_] Yes. If yes, how long have you slept? _____ minutes.
4. Have you felt hot flush through the day? [_] No [_] Yes.
   - If yes, how many? _____ At what time? _____

5. Have you been taken drugs, caffeinated coffee, caffeinated tea, cola, chocolate or alcohol today? [_] No [_] Yes. If yes, which ones, how much and at what time?

Name of the product	**Quantity**	**At what time**

6. Have you been outside today? [_] No [_] Yes. If yes, at what time? Did you wear sunglasses ? [_] No [_] Yes.

From (time)	**To (time)**	**From (time)**	**To (time)**

7. Do you experience pain or discomfort now? (i.e : headache, tingling, etc.) :

_____

8. Something happened to you today that you consider useful to report?

_____

9. Did you use an electronic devise (tablet, smart phone, television, computer, etc.) today? [_] No [_] Yes.
   If yes, which one(s) and at what time?

Devices used	**From (time)**	**To (time)**