EFFECTS OF AGE AND MOTOR TRAINING ON PREFRONTAL-MOTOR CORTICAL EXCITABILITY.

by

Susy Lam

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

© Copyright by Susy Lam, 2015
EFFECTS OF AGE AND MOTOR TRAINING ON PREFRONTAL-MOTOR CORTICAL EXCITABILITY.

Susy Lam
Master of Science
Institute of Medical Science
University of Toronto
2015

Abstract

The dorsolateral prefrontal cortex (DLPFC) is a critical substrate for motor learning. However, the role of the DLPFC in goal-directed, sequence-specific learning remains unclear, and the effects of age on motor learning and DLPFC-to-primary motor cortex (M1) connections have not been tested. We studied 14 right-handed healthy subjects, ranging from 20-80 years old, in a combined TMS-pinch grip experiment. The goals were to examine DLPFC-M1 connections in human subjects and how they change with motor training, whether sequence training changes M1 intracortical and DLPFC-M1 neurophysiology differently than motor training not containing a sequence, and whether age influences the TMS neurophysiological measures. All subjects were unable to regenerate the sequence, and behavioural/TMS measures suggest that they implicitly acquired the sequence. Sequence acquisition correlated with DLPFC-M1 facilitation. The results suggest that the brain processes sequence information differently compared to non-specific information during motor training. The DLPFC may play a role in processing sequence-specific information through an excitatory functional connection to M1 during acquisition.
Acknowledgments

Thank you to my supervisor, Dr. Robert Chen, for giving me this opportunity to become involved with translational neuroscience research,

To learn out of this journey that there is still so much more to learn.

Thank you to Dr. Michael Vesia and Carolyn for supporting me and providing guidance to me throughout this project.
# Table of Contents

Chapter 1: LITERATURE REVIEW

1.1: Introduction and Theories of Motor Learning ................................................................. 1
1.2: Different Stages of Motor Learning .................................................................................... 9
1.3: Methods to Study Motor Learning ..................................................................................... 16
1.4: Primary Motor Cortex ......................................................................................................... 21
1.5: Prefrontal Cortex ................................................................................................................. 28

Chapter 2: RESEARCH AIMS AND HYPOTHESIS .................................................................. 40

Chapter 3: MATERIALS AND METHODS

3.1: Subject Recruitment/Study Design .................................................................................. 47
3.2: Electrophysiological & Behavioural Methods .................................................................. 48
3.3: Magnetic Resonance Imaging and Neuronavigation ......................................................... 52
3.4: Analysis .............................................................................................................................. 56

Chapter 4: RESULTS .................................................................................................................. 64

Chapter 5: DISCUSSION ........................................................................................................... 88

Chapter 6: CONCLUSIONS/FUTURE DIRECTIONS .............................................................. 104

REFERENCES .......................................................................................................................... 106
List of Tables

Table 1: Active brain areas during early motor sequence learning ........................................7
Table 2: Calculating accuracy/response time skill measure ..................................................65
Table 3: Pearson correlations ...............................................................................................67
List of Figures

Figure 1: Modified diagram of Hikosaka’s Spatiomotor Conversion Theory ........................................7

Figure 2: Modified diagram of Luft & Buitrago’s Stages of Motor Learning........................................13

Figure 3: Study design ..........................................................................................................................47

Figure 4: Serial Visual Isometric Pinch Task ....................................................................................49

Figure 5: Time course of SVIPT task (trial-by-trial) ..........................................................................50

Figure 6: Coil positioning and electrode placement on hand muscles .................................................55

Figure 7: Brainsight marking setup ....................................................................................................57

Figure 8: Brainsight brain project exemplar .......................................................................................59

Figure 9: Schematic of behavioural measure calculations .................................................................62

Figure 10: Single pinch trial graph ....................................................................................................64

Figure 11: Response time data ...........................................................................................................70

Figure 12: Normalized response time data .........................................................................................71

Figure 13: Movement time data ........................................................................................................73

Figure 14: Group accuracy data ........................................................................................................74

Figure 15: Spatial error data ..............................................................................................................75

Figure 16: Normalized spatial error sample graph .............................................................................75

Figure 17: Skill measure (incorporates accuracy/RT) .........................................................................76

Figure 18: Random condition plot of MT and error rate .....................................................................78
Figure 19: Sequence condition plot of MT and error rate .................................................. 78

Figure 20: Skill measure (incorporates error/MT) ............................................................. 79

Figure 21: Baseline TMS data for single/paired-pulse recordings ..................................... 80

Figure 22: Baseline TMS data for dual site recordings ....................................................... 80

Figure 23: Single pulse TMS with training ...................................................................... 81

Figure 24: Paired-pulse TMS with training ...................................................................... 82

Figure 25: Dual-site TMS with training (90% RMT) ......................................................... 83

Figure 26: Dual-site TMS with training (110% RMT) ....................................................... 84

Figure 27: Correlations: Change in RT vs. age ................................................................. 85

Figure 28: Correlations: Change in SICI vs. age .............................................................. 86

Figure 29: Correlations: Change in DLPFC-M1 MEP vs. age ............................................ 87

Figure 30-31: Correlations: Change in DLPFC-M1 MEP vs. RT ....................................... 88

Figure 32-33: Correlations: Change in DLPFC-M1 MEP vs. spatial accuracy ................. 90

Figure 34: Correlations: Change in DLPFC-M1 MEP vs. skill measure ......................... 92

Figure 35: Cortico-motor/subcortico-motor pathways in DLPFC-M1 connectivity ........... 94
CHAPTER 1: LITERATURE REVIEW

1.1: Introduction and Theories of Motor Learning

It is important that we can reliably reproduce actions to accomplish the day-to-day routines in our lives, and this would not be possible without the ability to develop motor skills. Equally important is the ability to retain these motor skills for the long term; if retention was not possible, we would worry of accidents every morning when we start the car to drive to work, since we would always feel as if we were driving for the first time. To ride a bicycle, to write, to play a sport, without the ability to learn and retain motor memories, these actions would all seem novel to a person every time they engaged in the task. The fundamental process that underlies the acquisition and retention of these abilities is called “motor learning”, whereby specific movements are carried out more efficiently and accurately with practice. Motor skills that are learnt must be stable (i.e. resistant to interference) and adaptable.\(^1\)

Motor learning is a model for procedural learning, used to study cortical plasticity mechanisms, or how connections within the brain change and adapt to external stimuli. Procedural learning refers to the ability to gradually improve performance of a newly attained skill, commonly occurring over several training sessions\(^2\). The concept of motor learning is quite complex, but for simplicity and to provide a framework to build a meaningful understanding of motor learning, we can consider two main types of motor learning: motor skill learning and motor adaptations. Motor skill learning refers to being able to acquire new spatiotemporal muscle-activation patterns, such as when a musician learns a new set of finger motions for a new musical score, or when a person learns to combine a set of previously known movements together to form a new sequence of movements, such as serving a tennis ball. Motor adaptation comes in two forms: sensory-motor adaptation and conditional sensory-motor associations. Sensory-motor adaptations require subjects to modify already learnt movements to adapt to a new environment; for example, when an astronaut must learn to jump in microgravity. Conditional sensory-motor associations are similar to sensory-motor adaptations, with the exception that a person must learn to associate a specific movement or action with a particular “condition” or cue. An example of conditional sensory-motor associations is when a new driver learns, for the first time, to associate
the green-to-red traffic light change to switching their foot from the accelerator to the brake pedal. After this initial “conditional association”, subsequent actions of this type would fall under sensory-motor adaptation.

The result of being able to produce fluent, goal-directed actions is a result of interplay of complex neural networks, composed of both the cognitive and motor domains. Therefore, it is important to study procedural learning to gain a better understanding regarding mechanisms of how the human brain learns and retains skills, and the mechanisms involved when the brain becomes diseased.

**Theories of Motor Learning**

There are many theories of motor learning, which have been postulated for many decades. For example, Adams postulated a closed-loop theory of motor learning, where sensory feedback is used for the ongoing production of movement—errors in movement are detected through comparing the feedback of the movement to the memory of the intended movement\(^{255}\). Furthermore, Schmidt postulated a schema theory that is more abstract, emphasizing open-loop control processes instead, where motor programs do not contain specifics of a movement but rather general rules for different classes of movements\(^{256}\). As there are many theories of the motor learning processes, we will take a closer look at two important theories relevant to our study: Hikosaka’s Spatiomotor Conversion Theory and Willingham’s COBALT Theory.

**Hikosaka’s Spatiomotor Conversion Theory**

Hikosaka et al studied motor skill learning in macaque monkeys and humans in a trial and error button pressing task\(^3\), and found that subjects performed considerably better at old, but well-practiced (16 months old) compared to recent or very new sequences, as reflected in increased accuracy and faster reaction times. However, the human subjects reported not being aware of experiencing the old sequences, and both monkey and human subjects experienced faster reaction times in old vs. recent sequences, albeit having lower accuracy. Hikosaka went on to hypothesize that at least two neural mechanisms operate independently to represent a motor skill, and that the brain stores learned movement sequence through two domains: the motor information and the spatial information. These two types of information are stored through
activity of distinct neural circuits, where the spatial sequence is predominantly supported by the prefrontal-parietal cortical loops, and the motor sequence supported by the motor cortical loops.  

Figure 1 is an adapted figure based on Hikosaka’s review, and shows Hikosaka’s theory of motor learning. On the right side are the postulated processes and brain areas involved with learning a spatial sequence, and the left side for the motor sequence. Frontoparietal cortices form circuit loops with the basal ganglia and cerebellar associative regions, while the motor cortices form circuit loops with the motor regions of the basal ganglia and cerebellum.

When learning first begins, movements are executed through the “spatio-motor” conversion process, where the initial associations between the spatial cues and the motor movements are being formed (indicated by horizontal connections). After learning, the spatial sequence is supported by the parietal-prefrontal cortical loops and the motor sequence supported by the motor cortical loops (vertical connections). It is further hypothesized by Hikosaka et al that motor sequences are more slowly acquired, and are processed implicitly, whereas spatial sequences are usually processed explicitly, and hence are more quickly acquired. Long-term retention of a motor sequence is thought to be mainly attributed to the motor sequence mechanism, such that the sequence and its speed can be maintained, even without awareness or attention demand. The spatial sequence mechanism, on the other hand, is much more demanding of attention.
Figure 1: The figure above is adapted from Hikosaka et al’s review and postulated theory of motor skill learning. The blue boxes on the left indicate hypothesized areas important for the process of learning a spatial sequence, i.e. the parietal-prefrontal cortical loop; the green boxes on the right indicate areas important for learning a motor sequence. There is a crossover from the spatial sequence learning loops to the motor sequence learning loops at the premotor areas/SMA.

Willingham’s COBAL T Theory

Willingham proposed a useful neuropsychological theory that provides insight regarding the components of motor skill learning. That is, learning a new skill grows directly out of motor control processes and the various processes that support motor control become tuned to a particular task with time, and therefore will operate more efficiently. This theory, the control-based learning theory, or COBALT, uses three motor control principles in its operations: (1) neural separability principle, (2) disparate representation principle, and (3) dual mode principle\(^1\).

The first principle, neural separability, postulates that the different anatomical areas of the brain are responsible for processing and executing the different cognitive components of motor
control—some of these processes including the perceptual motor integration process, sequencing, innervation of the correct muscles for a particular goal-directed action. An example is the role of the dorsolateral prefrontal cortex (DLPFC). There have been many studies since the 1970’s that describe the DLPFC as being important for encoding movement planning in an environment in terms of behavioural significance or reward\textsuperscript{5,6}. Patients with frontal lobe damage show abnormalities and errors in computing behavioural goals, such as putting a piece of string instead of pasta into a pot. It was first seen in these studies that lesions of this type more often led to these deficits in goal-directed behaviour\textsuperscript{7}. From examples such as these, according to the principle of neural separability, have paved more evidence that suggest specialized roles for different anatomical brain areas, such as the supplementary motor area as being more important for internalized, well-practiced actions.

The second principle, the disparate representation principle, indicates that there are three separate representations in motor control: for the strategic process, an allocentric space for goal selection; in the perceptual-motor integration and sequencing process, an egocentric space for target selection; and for the dynamic process, the muscle innervation for movement to occur. Allocentric refers to the spatial frame of reference in which an object location is coded relative to another object, and egocentric is when object locations are coded relative to some part of the body, such as the hand or head. Essentially, this second principle delineates what components are required towards successful execution of a motor plan. First, the target or object in an environment is identified as the goal; then, neural processes integrate the perceptual domain with the motor domain, putting together a feasible motor plan; and finally, the effector muscles execute the movement in a properly planned manner in order to reach the goal. As a result, different brain areas work in concert to allow for a multistep process eventually leading to successful movement. That being said, if specific neural substrates that are involved with particular encoding steps of this process are damaged, this can have a major effect on the subsequent final execution of the movement. For example, damage to areas involved with visual perceptual encoding, such as the temporal cortex, causes patients to complain of limited perception of objects and visual impairment, even though they were able to show normal motor behaviour, being able to grasp an object and make visually-guided eye movements, despite not being able to describe and distinguish objects according to features such as size and shape. On the other hand, patients who have damage to the posterior parietal cortex were not aware of
impairment of perceptual awareness of the target object, but when attempting to reach the object, they are severely impaired (reviewed in Chapter 4 of Milner & Goodale, 1995). Single-cell recording studies support the lesion studies, in finding that cells in the temporal cortex are object-centered and code objects allocentrically where cells in the posterior parietal cortex code space egocentrically.

The third principle, the dual mode principle, postulates that all voluntary actions are initiated by a conscious environmental goal. Subsequent transformations, such as perceptual motor integration, movement sequencing, and dynamic muscle innervation, generate representations for the movement and are done so outside of awareness. Thus, a conscious strategic process is thought to be attention-demanding, where the environmental goal is selected; the subsequent processes are unconscious less attention demanding. The latter process can also be attended to by the subject consciously, but it will recruit different brain processes. For example, the prefrontal cortex is activated during a finger sequence learning task, during the initial learning stage, but not when the sequence was well practiced. However, when subjects were asked again to attend to their performance, the prefrontal cortex was reactivated. Thus, the third principle of COBALT sets the stage for either unconscious or conscious motor execution processes, only after the environmental goal is consciously determined.

According to COBALT, there are two mechanisms in this framework that support motor skill learning. Firstly, the perceptual-motor integration, sequencing, and dynamic processes become more efficient with training for a particular task, with the processes becoming more tuned to the task, allowing for behaviourally observed increases in accuracy for a particular movement. Secondly, the strategic process, which is not as tuned as the other processes are, may contribute to improved behavioural performance either by picking more effective environmental goals, or by sequencing more effective targets for movement, under the conscious state.

Next we will discuss how the COBALT theory can aid in explaining a specific learning paradigm, pertaining specifically to a task that will eventually be relevant to our study, which is the serial response task, such as the SRTT. The SRTT is a task developed originally to test the attention requirements of learning by Nissen & Bullemer. Subjects are required to press a series of four buttons with fingers of one hand, each button of which corresponds to a specific bar cue.
on the screen. When subjects learn to associate different spatial cues to pressing different buttons with their fingers in serial sequence, their response times when the cues appear in sequence are decreased compared to when cues appear in random order, and this is a measure of sequence learning. If a subject engages in the task long enough, they may eventually become consciously aware of the sequence and explicitly memorize it, through which the strategic processes will contribute to the behavioural improvements at this stage. During the implicit stage, however, the conscious, strategic process is only engaged at the beginning of each trial, where the subject determines the target on the computer screen. The remainder of the trial depends on conditional sensory-motor adaptation, where the subject learns to associate a particular finger with a particular target cue, through engaging the processes of perceptual-motor integration (seeing a particular bar flash on the screen as needing to engage the index finger), sequencing (less relevant in this example, but more relevant when putting together a series of pre-learnt movements repeatedly), and dynamic learning (engaging the different muscles involved in pressing the index finger-associated button and releasing it). Overall, the COBALT theory is a useful framework to consider when thinking about motor learning and how to associate various learning paradigms to motor learning. Next, we will discuss the various stages in motor learning and the neural networks engaged.
1.2: Different Stages of Motor Learning

Learning proceeds through various observed stages, and the improvements from these trainings are reflective of changes in the underlying brain neurophysiology, due to the establishment and stabilization of the motor memories formed\textsuperscript{13}. It is interesting to study how motor memories are formed and stabilized in the process of mastering a skill, and one of the ultimate goals for neuroscientists who study motor learning is to delineate the neural circuits involved and how they contribute to the complex processes of motor learning and memory formation mechanisms.

Since motor tasks can vary in difficulty, the time it takes to master them also varies. It is common to see significant improvements within the first session of motor training, while more complex skills can require multiple sessions for the same degree of improvement. The gauging of what tasks are easier versus harder is complex to quantify, and also depends on the participant’s level of expertise in the task. It may take a novice many weeks to learn how to play Debussy’s Arabesque No. 1, but a professional musician is able to sight-read and learn a piece significantly quicker. It is also much more difficult to master a motor skill through one extensive training session; rather, spacing out different training session intervals reaps greater improvement\textsuperscript{14,15}. Normally, the performance at the end of one session is not maintained through to the beginning of the next session—that is, the subject usually begins the subsequent session performing at a slightly worse performance, but eventually improves further than where they had left off in the previous session. This phenomenon is called the “warm-up phenomenon”\textsuperscript{16} (See Figure 2).
Figure 2: The above figure is an adapted, simplified figure from Luft & Buitrago, 2005, with evidence suggesting that motor learning proceeds through various stages through which different methods of acquisition, storage and active substrates predominate.

The transition from the initial motor task exposure to ultimate mastery proceeds through three main stages: intra-session (fast) learning, consolidation, and inter-session (slow) learning, while interference is a phenomenon that serves to test the stability of newly-learned motor memories. Fast learning refers to an improvement in task performance, readily observed within the first training session. Thus, it is also commonly referred to as “within-session learning”. Between-session learning, on the other hand, is also called “slow learning” and refers to the improvements in behavioural performance observed over multiple training sessions. The improvements from slow learning are much less obvious than in within-session learning, as the benefits in slow learning have reached a plateau or “ceiling” stage, and subjects are nearing their peak of motor performance. Finally, memory consolidation is a concept conceived by Muller & Pilzecker in 1900, and refers to the stabilization of memories over time, reducing their vulnerability to interference. We will now explore the different stages of learning that were defined and the various studies that were conducted to elucidate the brain systems involved.
**Time course of Changes during Motor Learning**

Through this section, we will first compare research evidence suggesting that specific neural substrates are preferentially active during the different stages of motor learning (fast learning, slow learning, consolidation, interference).

**Fast Learning vs. Slow Learning**

There are varying opinions regarding which brain areas are activated or suppressed during the different stages of learning, as evidenced in neuroimaging studies of motor learning. Some studies report activation during sequence learning, whereas others report deactivation (see Table 1 for a compilation of studies that showed various brain areas that become activated in the early stages of learning, or the “fast” learning stage). In neuroimaging studies by Jenkens et al (1994) and Jueptner et al (1997), subjects were given the task of learning a sequence, and hence, because subjects were saliently aware that they were to learn one, the learning is deemed “explicit” learning\(^{11,19}\). These studies compared brain activity when learning an entirely new sequence, versus a previously learned sequence (PRESEQ), thought to have had this memory stabilized because subjects could perform well on the PRESEQ while engaging in a secondary task, with minimal interference. The studies found that when learning a new sequence, there was more activity in the prefrontal, anterior cingulate, parietal, and premotor cortices, compared to PRESEQ. In contrast, the supplementary motor cortex (SMA) was more active during PRESEQ.

In contrast, Grafton et al performed a similar study, but reported different findings. Grafton et al found that with learning, there were increases in activity in the putamen, SMA, and the sensorimotor cortex\(^{20}\). If subjects eventually became aware of the sequence, there were correlated increases in prefrontal, premotor, and parietal cortices activities. However, the differences in the results in this study and the previous studies could be due to differences in methodology. Learning was implicit in part of Grafton et al.’s study, as opposed to explicit, since subjects were not instructed to expect a sequence. This meant that these subjects were not aware that they were learning a sequence as they were conducting the task. Similarly, Toni et al.’s research group aimed to study the pattern of brain activity during the entire course of learning, from implicit to explicit recognition of the sequence. They reported a fast reduction in errors early in sequence learning, but reaction time did not show any consistent patterns with training.
Increases in brain activity were noted in the right dorsolateral prefrontal cortex and left dorsal premotor cortex in early learning, followed by decreases after acquiring the sequence. In contrast, the SMA experienced increases in activity in later stages of learning\(^21\).

Floyer-Lea & Matthews (2005) used fMRI to study the different regions of the brain activated at different stages of motor learning, using an isometric force production pinch task with the hand to track a cursor in 8 second intervals\(^22\). This study found that short-term learning was associated with initially increased activation, and progressively decreased activation of the dorsolateral prefrontal cortex, anterior cingulate cortex, primary motor cortex, posterior parietal cortex, and cerebellar cortex with training. On the other hand, increased activation was observed in the right cerebellar dentate gyrus, left putamen, and left thalamus. These findings are in keeping with the theory that different stages of motor learning are governed by different neural substrates, where short-term learning is associated with activation of cortical networks specific for learned movements, and long-term slow learning is associated with increased bilateral activation of a cortical-subcortical network, suggesting that there is a plastic development of representations of both the motor output and the somatosensory afferent information\(^22\).

<table>
<thead>
<tr>
<th>Authors</th>
<th>Type of Imaging</th>
<th>Motor Cortex</th>
<th>Parietal Cortex</th>
<th>Prefrontal Cortex</th>
<th>Cerebellum</th>
<th>Basal ganglia</th>
<th>Dorsal Premotor Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghilardi et al. (2000)(^{23})</td>
<td>PET</td>
<td>N/A</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Toni et al. (1998)(^{21})</td>
<td>fMRI</td>
<td>N/A</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Floyer-Lea et al.</td>
<td>fMRI</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Jueptner et al (1997)(^{11})</td>
<td>PET</td>
<td>---</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Grafton et al (1995)(^{20})</td>
<td>PET</td>
<td>---</td>
<td>↑</td>
<td>↑</td>
<td>---</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Sakai et al (1998)(^{24})</td>
<td>fMRI</td>
<td>N/A</td>
<td>---</td>
<td>↑</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Doyon et al (1996)(^{179})</td>
<td>PET</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>---</td>
<td>↑</td>
</tr>
</tbody>
</table>
Table 1: The above table shows which brain areas are active during the early stages of spatial motor sequence learning (spatially segregated & distinct targets for each effector finger) compared to the later stages (where a skill is overlearned, explicit). Upward arrows indicate an increased activation during early learning stages, dashes indicate that an area is not more active during early learning. N/A indicates that the area was not reported in the study. Downward arrows indicate decreased activation during early learning stages.

To extend this further and illustrate the pattern of brain activation throughout motor learning, Sakai et al used a sequential button pressing task in human subjects to show that activation during motor skill learning proceeds from the frontal to parietal areas\textsuperscript{24}. Through functional imaging analysis, Sakai et al showed that the dorsolateral prefrontal cortex and pre-supplementary motor area are activated during early stages of learning, whereas areas that were more parietal, such as the interparietal sulcus and precuneus, were activated later in motor learning. Petersen et al and Toni et al also reported similar dynamic changes using functional brain imaging\textsuperscript{21,25}. Furthermore, Honda et al\textsuperscript{26} reported that explicit knowledge of a motor sequence activated the prefrontal cortex, but not the sensorimotor cortex.

Learning of the substrates involved during the different phases of motor learning is an important building block towards appreciating the relations between brain mechanisms and behavioural performance. In the next sections, we will review literature pertaining to studies that explored other processes that occur during motor learning, and the neural substrates that are implicated.

Consolidation

Consolidation refers to a process of stabilizing memory traces after its initial acquisition, and is a process of learning that has been extensively studied in animals. For example, Andalman & Fee\textsuperscript{27} studied songbirds and discovered evidence that suggests that the anterior forebrain pathway (AFP) provides instructive input to improve performance of the motor pathway, to avoid vocal errors, suggesting that the AFP pathway actively drives plasticity in the motor pathway of these birds and partakes in the consolidation process. Likewise, Yin et al\textsuperscript{28} studied the striatal circuit of mice as they acquired and learned a motor skill during a rotarod task, and using in vivo recordings, they observed region-specific changes in neural activity during the different phases of skill learning, with the associative and dorsalmedial striatum being preferentially engaged in
early learning, while sensorimotor and dorsolateral striatum were engaged later in learning. Using *ex vivo* recordings, they were able to record from medium spiny striatal neurons in brain slices of trained mice—which showed training-specific changes in the excitatory synaptic transmission of the striatum. Essentially, Yin et al.’s findings provide evidence supporting a region-specific and pathway-specific long-lasting synaptic plasticity in the striatum when mice learn and consolidate a skill. Therefore, this evidence strongly supports the crucial role of the striatum in skill learning and consolidation. Other studies in rodents have also supported for differential corticostriatal plasticity during fast versus slow motor skill learning in mice\textsuperscript{29}, and the transient spine expansion and learning-induced plasticity in layer I of the primary motor cortex\textsuperscript{30}. Lesions in the striatum have also been shown to disrupt motor skill acquisition and retention in mice, but this does not disrupt implicit learning\textsuperscript{31}.

Studies regarding consolidation have also been tested on human subjects. Walker and colleagues investigated healthy subjects who performed a sequential finger tapping task, and discovered that those who had a period of sleep between training sessions experienced additional performance gains after sleep\textsuperscript{32}. Furthermore, Reis et al. have shown that non-invasive brain stimulation can enhance motor skill acquisition over the course of multiple days through a positive effect on consolidation\textsuperscript{33}. In this study, transcranial direct current stimulation (tDCS) was delivered to the primary motor cortex while subjects practiced a sequential spatial pinch task with their hand. There was greater skill acquisition both within a short term (few days) and retention of skill at the 3-month follow up period with tDCS. These results suggest that the primary motor cortex plays a critical role on motor skill consolidation, and that brain stimulation can help augment this process. They have implications for brain injury rehabilitation. Functional neuroimaging studies also show that after a period of six hours after learning a motor task, new brain areas were engaged to perform the task compared to baseline, wherein there was a shift from prefrontal regions of the cortex to posterior parietal, premotor, and cerebellar structures\textsuperscript{34}. These findings suggest that over practice, there is a change in neural representation of the motor skill being learnt, and these neural changes may reflect increased functional stability of the encoded motor memories. Aside from the duration of training affecting the rate of learning, the planned intervals of when training sessions occurs also plays a role. Therefore, learning strategies influence the rate of memory consolidation. There is evidence that spacing out training sessions, with planned breaks in between, reaps a greater degree of improvement compared to a continuous session.
Notably, factors that can influence the degree of improvement between sessions include sleep and mental rehearsal, and studies such as Walker et al.’s study provide evidence that the motor system is able to undergo a form of “rehearsal” or consolidation mechanism during sleep that aids in the subsequent behavioural performance.

However, some studies argued that the influence of sleep on motor skill consolidation is not significant. Nemeth et al investigated implicit motor learning in young and old adults before and after an offline interval of 12 hours, which either included or did not include sleep. Results from this study showed that general skill learning after the offline state improved in both the younger and older groups, with the young improved more than the old. However, the improvement was not sleep-dependent, and thus they concluded that implicit motor sequence learning of this kind may not be influenced by sleep\textsuperscript{35}. Overall, evidence regarding the role of sleep in memory consolidation is mixed, and requires further study.

**Interference**

Interference is the process of learning a new task, which subsequently affects performance of a previously learned task. It is assumed that learning the new, interfering tasks consumes the same resources in the brain as when learning the old task, and this phenomenon is commonly used in motor learning studies to provide insight into the stability of learned motor memories. A study conducted to track a moving object in space with prism goggles that distort and invert vision, is an example of how using interference can provide insight to which types of information processes are important at different stages of motor learning\textsuperscript{36}. In this study, Eversheim et al used a dual task study: either an attention-demanding task or a visuospatial transformation task along with tracking a moving object in space using inverted goggles. This study found that the attention-demanding condition only interfered with the performance in the visual tracking early in the task, while visuospatial transformation condition affected tracking performance only late in learning. Other studies have also explored learning consolidation using interference. Studies that used a secondary task to interfere immediately after learning a primary task show impaired consolidation\textsuperscript{37}, while consolidation is not affected if the secondary task is introduced 4-5 hours after learning the primary task\textsuperscript{17}. Walker et al demonstrate in this study that a consolidation period between 10 minutes to 6 hours after learning the initial task provides resistance against
interference when introducing the subsequent secondary task, shedding light into an ongoing consolidation process occurring.

**AGING AND MOTOR LEARNING**

Aging is defined as an inevitable, complex, multifactorial process wherein there is a gradual deterioration of organ systems and tissues over time. It is influenced by factors such as gender, genetics, radiation exposure, diet, and exercise, where two individuals who are the same chronological age may differ both in physiological state and in physical appearance, due to these factors. It is generally accepted that the aging process is compartmentalized into three categories: cellular/homeostatic mechanisms, decrease in organ mass, and finally a decrease in the overall functional reserve of the body’s systems.\(^{245}\) We will discuss mainly age-related changes in the brain structure, cognition, and functional connections during motor learning.

Longitudinal studies of brain volume changes using MRI have shown that aging significantly decreases the global brain volume, particularly in the temporal lobes and frontal cortices.\(^{246,247}\) Furthermore, pertaining to the motor cortex, Nakamura et al.\(^{248}\) reported that the horizontally-oriented dendrites, or basal dendrites of layer V pyramidal cells in the human motor cortex, significantly decrease with aging, and other groups also reported age-related decreases in dendritic spines of layer III pyramidal cells in the human motor cortex. Thus, in aging, there are irreversible deteriorations occurring in the motor cortex and also in areas of the cortex associated with cognition, ultimately taking a toll on functions such as attention, information processing, working memory, and motor learning. Damoiseaux et al.\(^{249}\) found reduced resting state brain activity in the “default-mode network” during normal aging, where nodes of this default network (comprising the superior and middle frontal gyrus, posterior cingulate, middle temporal gyrus) show decreased activity that is correlated with impairment of executive functions and information processing speed in the older group. Overall, age-related changes in brain structure ultimately have impact on the functional connections between different brain regions in various networks. Another example is where Bennett et al. reported in a DTI imaging study that decreases in white matter integrity in the tract between the dorsolateral prefrontal cortex and the caudate nucleus of the basal ganglia was associated with worsened performance in implicit sequence learning, and that age was strongly associated with these white matter changes.\(^{200}\) It is
interesting to study how these functional connections change with aging, and how age-related changes in these functional connections impacts motor learning.

1.3: Methods to Study Motor Learning

Behavioural Methods

Learning motor skills requires involvement of multiple modalities, including, but not limited to, visual perception, decision-making, and motor execution. These are thought to be primarily operated through a series of neural networks, and various approaches have been used by scientists to study these components of motor learning as separate entities, in efforts to more precisely characterize their research questions.

Examples of tasks that embody these approaches include the serial reaction time task (SRTT), where subjects typically use their fingers to respond to a series of bars on a computer screen, where each bar corresponds to the button each finger should press. This paradigm was originally developed by Nissen & Bullemer (1987), and learning in this task results in faster response times when subjects are exposed to a repeated sequence of cues rather than random ones. Tasks like this aim to measure how subjects’ responses change when engaged in a motor learning task improve with training and practice.

Another task that is used often to study motor learning is a joystick manoeuvring task, where subjects control a joystick with their hand to reach specific co-ordinates that they associate with as the targets. In these visuomotor tracking tasks, subjects use more of their wrist muscles in order to increase their trajectory accuracy. Similarly, joysticks can be used to trace the pattern of a moving sinusoidal wave, and the improvements in accuracy can be used to gauge motor skill. This sinusoidal tracking task goes back as far as 1899, when Woodworth was investigating line-drawing tasks and reported irregularities in the line trajectories just before reaching the target. Woodworth postulated that this irregularity was a “signature” of a corrective control phase that reduces errors in reaching the target during the trajectory. Since Woodworth, investigators have focused on gaining insight into the nature of the motor control processes involved in generation of skillful movements.
Tests of sensory-motor adaptation cannot prove that motor learning had occurred, because there is the confounding factor of motor adaptation that may not actually alter the underlying cortical circuits. However, if there is originally no association at first between the given sensory cue and the resulting motor movement, this can be tested and shown at baseline, and subsequent tests with practice can show that there is an improvement from baseline, and that the brain has actually learned to associate an irrelevant sensory cue with a motor movement and has improved on it in various dimensions.

**Brain Imaging**

In addition to these behavioural tasks, there are methods that can quantify changes in the brain, including the sizes of brain substrates and tract fibers, or the on-line activation of specific brain areas in response to specific stimuli. Commonly used techniques to measure brain activation and changes in activation size of various brain areas is positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). It is common that a combination of behavioural paradigms and neuroimaging methods are integrated together in studies in order to measure the on-line and off-line effects of motor learning.

**Transcranial Magnetic Stimulation**

Another method to measure changes in motor circuitry is transcranial magnetic stimulation (TMS), which is a non-invasive brain stimulation technique that delivers magnetic pulses through the skull to excite neurons in the brain. Delivering single pulses to the primary motor cortex and measuring the subsequent motor-evoked potentials (MEP) at different stages of motor training can provide insight on the changes in excitability of the motor cortex. Studies have also shown that providing stimulation to the median nerve can alter motor cortex excitability. This is because activity of pyramidal tract neurons of the motor cortex changes in response to peripheral stimulation, as evidenced in non-human primate studies as well as human studies, where facilitation or inhibition of the motor-evoked potential can be elicited depending on the onset between peripheral nerve stimulation and the TMS pulse.

Patterning TMS pulses of varying magnitudes and pairing them in different intervals modulates the resulting firing activity cortical neurons, either increasing (facilitation) or decreasing
(inhibition) their activity. Certain measures, such as short intracortical inhibition (SICI), there is consensus that it is an estimate of the excitability of the post-synaptic gamma-aminobutyric acid A (GABA-A) receptors in the primary motor cortex. TMS has been therefore been extensively used to study motor learning by examining changes in pre-determined TMS measures associated with training in a particular motor task; for example, it is commonly seen with motor training that there is a decrease in SICI, indicating that modulation of SICI is involved in the process of motor skill acquisition, and hence that improvements in behavioural skill measures were associated with reduction of inhibition. Furthermore, these results support SICI as playing a role in the mechanism driving use-dependent plasticity. However, studies have argued that changes in intracortical excitability may not be essential to skill retention.

Complications of Electrical/Magnetic Stimulation

One of the more complicated issues in studying the brain using electrical or magnetic stimulation is approximating the site and region stimulated. The act of providing an electrical or magnetic stimulus creates effects on neural transmission that are still not entirely clear. For example, what is known about intracortical stimulation in MI is that it activates pyramidal tract neurons that ultimately produce muscle activity with the temporal and spatial summation. However, electrical stimulation also activates intracortical dendritic and axonal processes around the stimulation site, and perhaps most significantly, stimulation also activates the recurrent axon collateral systems for each neuron brought to threshold. Many cerebral cortical pyramidal projection neurons have local collateral branches, in addition to the efferent axon. The branches synapse in the immediate vicinity of the neuron and typically have substantial horizontal, or lateral, connection systems that can extend upward of 1 cm within M1. Thus, one must acknowledge that projection neurons not only participate in the internal processing of M1; they also relay the output message to subcortical systems.

Finally, electrical stimulation of pyramidal tract neurons sends signals not only to the spinal cord but also to a vast set of supraspinal, subcortical targets, with the brainstem, striatum, and thalamus. All of these factors should be taken into consideration when interpreting results using electrical and magnetic stimulation to study the motor cortex and associated areas.
Animal Studies of Motor Learning

Studying animals proves to be useful to determine the mechanisms of motor learning and memory. Paradigms that were developed to study motor learning in these animals include two main approaches: (1) tasks that improve naturally expressed motor behaviours and skills, and (2) reward-based learning to train new motor sequences. Fundamentally, all behavioural studies that require the animal to repeatedly produce a desired response will have some aspect of motor learning incorporated into them.

Initially, Hebb postulated the idea that memories are encoded at the synapses and that changes at the synaptic level constitute the basis of learning and memory. Since then, there have been a wide variety of studies on the biochemical level on the effects of learning on the ensuing brain physiology. Motor learning has been thoroughly studied in animals, such as rodents, birds, and non-human primates. In animal models, it is difficult to separate declarative and procedural learning, since one cannot determine whether an animal can recall a specific memory, and can only study animals through their behaviour. However, the behavioural learning curves of spatial learning, such as the water maze task for rodents, are not substantially different from that of skill learning.

Despite rodents lacking development in certain aspects, such as specialization for manual dexterity and visuomotor skills, they possess basic mammalian neural systems for motor control similar to that of humans, including a descending motor pathway from the cortex to brainstem and spinal cord, along with feedback pathways through the basal ganglia and cerebellum. Thus, overlapping similarities in their nervous system compared to humans drives the impetus to study motor learning using these models. One frequently used and reproducible skill learning paradigm in rats is the forelimb reaching task, where rats learn and refine a complex forelimb reaching movement to reach food pellets, but prior to mastering this reaching, the rat must learn the position of the pellet in space, and the concept of using its force limb in a particular manner to retrieve the award—this process is comparable to our learning paradigms.

The hypothesis behind earlier studies is that consolidation requires modifications in neuronal circuitry, and thus a plastic change in their structures. Thus, newly expressed proteins are thought of as serving the purpose of building these new structures during motor learning. Thus,
many studies have investigated the effects of inhibiting protein synthesis on the subsequent effects on motor learning. It was found that using a protein synthesis inhibitor on mice that were trained to perform an acrobatic motor task were impaired in their performance not during learning, but between sessions of training. This suggests that protein synthesis is required for motor learning processes in consolidation, between motor training sessions, such as during rest. To further localize the substrate involved in this consolidation process, Buitrago et al. injected the protein synthesis inhibitor into the primary motor cortex, the cerebellum, and the parietal cortex of mice that learned this task. Learning between sessions was only affected in mice that were injected in the primary motor cortex. This provides strong evidence that the primary motor cortex is a critical substrate for consolidation during motor skill learning, and that protein synthesis is necessary in the primary motor cortex after training in order for successful motor learning to occur. Of importance is that motor skill learning in animal models has been shown to have fast and slow learning components as well.

Different areas of the brain have been shown by researchers to be active during different stages of motor skill learning in animals. In a rodent study using multi-electrode recordings in awake-behaving mice, Costa et al. discovered that improvements in performance on an accelerating rotarod task correlated with substantial plastic changes in the motor cortex and the striatum, where a high percentage of striatal and motor cortex neurons were “task-related” (in other words, they fired only when the mice ran on the rotarod). The “latency-to-fall” skill measure in mice had improved significantly during the first training session, correlating well with the parallel recruitment of task-related neurons in striatal and motor cortical regions. On the other hand, during slow learning, there was a refinement of firing patterns in each structure. This study demonstrates that there is a difference in task-specific neuron recruitment between fast learning and slow learning, suggesting that there are different cortical plasticity mechanisms at work for these two different types of learning. It is argued that distinct neural mechanisms are at work during these two different learning stages.

Overall, there are many methods to study motor learning in mammals, and these paradigms have paved and shed light on approaches to study motor learning in human subjects. The proceeding section will discuss more in depth regarding the primary motor cortex, its structure and function, and its role in motor learning.
1.4: Primary Motor Cortex

The primary motor cortex (M1) is located in the posterior portion of the frontal lobe, defined as Brodmann area 4. M1 is an intriguing cortical area, working together with motor, motor association, and subcortical brain regions to plan and execute movement. Aside from having a diverse range of anatomical connections to and from other cortical and subcortical areas, M1 also has extensive corticospinal projections to the effector muscles, and hence is a direct communicator to the effectors. Within the past decade, there have been crucial paradigm shifts pertaining to understanding how motor cortical areas function as information processors to plan and control movements. Recently, evidence has also surfaced supporting their roles in higher-order processes, such as learning and cognition. Research on M1 alone has sparked significant discussion regarding the advances of this area, and its role in functional, plastic organization in higher-order mammals, its ability to change synaptic strength based on degree of activity, its important role in controlling muscles and movement, and finally, its crucial role in motor learning.

Functional Organization

A previously dominant and popular notion was that the primary motor cortex is that it is organized in an exceptionally detailed manner, deemed the “homunculus”, or a somatotopically ordered representational map for muscles and movement, which was originally represented as a distorted cartoon of the body\(^ {77,78} \). The mapping of the motor cortex was conducted using low-intensity electrical stimulation. The main idea was that the map consisted of a medial-to-lateral representation from leg, to arm, head, and face, and this notion perpetuated the early concepts of motor cortex organization: that representations for each body part are orderly and occupy cortical space in a non-overlapping manner, and that specific elements, such as a cortical column, is responsible for controlling a dedicated body part, such as a sole finger\(^ {79} \).

Despite the initial evidence that supported this idea of a homunculus, early doubts were raised that this somatotopic map existed at a level of high detail. More recent evidence is in keeping with M1 displaying functional subregions for rough body parts such as arms, legs, and head, but refutes this idea of a detailed somatotopic map. Instead, more evidence is leaning towards M1 having overlapping representations, consisting of networks involving large neuronal populations
within a subregion\textsuperscript{80}. Furthermore, an influential research method adapted by Stoney et al.\textsuperscript{81} used microelectrodes to focally stimulate cortical neurons by inserting the electrodes to these neurons, and moving the electrodes in small steps. This group found that new movements were evoked even from small steps; however, when these movements were assembled into a map, the sites of stimulation for any body part appeared to be widely distributed, redundant, and overlapping, and these were confirmed in subsequent studies\textsuperscript{82–86}. This method provided a higher resolution functional map of the primary motor cortex.

Further studies using neural recordings supported the notion that single neurons in M1 influence multiple arm muscles\textsuperscript{87,88} and that they participate in multiple hand motor actions\textsuperscript{89}. Studies using focal inactivation of M1 in non-human primates also show that arm actions are affected on a more global scale as opposed to focal; yet the effect on moving individual components is unaffected\textsuperscript{90}. Evidence from these studies contradicts the original concept of a non-overlapping motor homunculus, and elucidates a more encompassing role of individual M1 neurons in motor control.

Results from advanced neuroimaging methods also support the new-born idea that M1 functions are distributed throughout subregions. High-resolution functional neuroimaging has revealed that activation within M1 of the wrist, elbow, and fingers overlapped in area, consistent with the overlapping theory\textsuperscript{91}. Further studies looking at connectivity between M1 arm and the fingers show no clear topographic plan for its horizontal connectivity with fingers, wrist, or other arm components\textsuperscript{64}. These experimental results all point towards a pattern of M1 being organized in gross subdivisions, but within each subdivision, there is an internally distributed network that exerts motor control in a broad pattern of activity\textsuperscript{43,80}. These results elucidate that perhaps M1 is organized in this manner to be modifiable and flexible for learning and cognition.

The primary motor cortex is dynamic and plastic

Although many invertebrates with less-developed nervous systems are able to display complex motor skills, there is no other organism of this classification that can match the abilities in skill, adaptability, and precision of motor control in organisms that have evolved large forebrains. The idea of having a dynamic organization in M1 has been an old concept\textsuperscript{92}, and recent studies that examined intracortical mapping via electrical stimulation have demonstrated that maps within
M1 are capable of fast and enduring reorganization. Experimental transection of a facial motor nerve, rendering mice unable to functionally move their whiskers, allowed for replacement of the part of M1 responsible for whiskers by the adjacent forelimb and eye. Amazingly, this reorganization occurred just hours of the nerve transection, and this reorganization persisted for months. Sanes et al. concluded that because the excitability threshold for the reorganized area and normal areas of M1 are similar, this reorganization represents an expansion of a still-functioning representation to replace a part that no longer functions.

Aside from animal studies, more current research methods have allowed for human motor cortical reorganization to be studied via non-invasive brain stimulation. Movement representation maps have been shown to be modified by different changes to M1 input, such as amputations, transient changes in sensory inputs, spinal cord injury, and non-invasive brain stimulation. Consistency of results through a diverse range of experimental approaches solidifies the notion that the primary motor cortex is dynamic and adaptive in nature, providing motor maps for higher order organisms with more developed forebrains.

Now that it is understood that M1 is capable of reorganization, it is also important to discover what is responsible for this ability. The likely substrate responsible for mediating M1 plasticity is the system of horizontal fibres that span the motor cortex. A study by Jacobs & Donoghue (1991) unravelled the existence of these subtle motor maps in M1 through blocking GABAergic inhibition. Through blocking the function of these inhibitory receptors, the horizontal connections were discovered, which were thought to be normally blocked by feed-forward inhibition. Slice preparations of the motor cortex have also shown that these excitatory horizontal fibers are mediated by glutamatergic receptors, and that feed-forward GABA inhibition likely is responsible for regulating excitation strength. These results shed light on two important ideas: that M1 consists of intrinsic circuitries that are important to support its own reorganization, and that the nature of reorganization within M1 is likely dependent on the exact balance of excitatory and inhibitory influences within the M1 network connections. As mentioned briefly earlier, Huntley et al.’s work on facial nerve lesion of rats demonstrated that significant reorganization of M1 occurred predominantly at the M1 area responsible for moving whiskers, but not at other M1 zones. This significant finding through electric stimulation mapping echoes the evidence of a horizontal network connection within M1, and the
reorganization of these connections can indeed be reflective of synaptic plasticity of these fibers.101

Dynamic, moment-to-moment modulation of functional organization within M1 can be mediated by these horizontal fibers, yet another question more pressing and relevant to motor learning is whether and how changes in efficacy of these connections occur, and how a stable form of synaptic modification can be achieved. Literature regarding activity-dependent synaptic plasticity is well-documented for M1.102,103 The horizontal neuronal connections in M1, therefore, may mediate the generation of new associations between populations of M1 neurons, therefore having the capacity for long term synaptic modification.100,104 Similar to the hippocampal collateral system, and also in the neocortex during development, M1 plasticity is N-methyl-D-aspartate (NMDA) receptor dependent, providing clues that the chemical substrates responsible for orchestrating synaptic plasticity are similar, at least within these two structures, and also that synaptic modification and plasticity continue within the adult cortex. It is worthwhile to note that induction of LTP and LTD has been explored in the literature, with LTP in the mature M1 appearing to be more constrained, in the sense that LTP cannot be induced by stimulating the horizontal M1 pathways alone, unless inhibition is transiently reduced during this induction.100 LTP is best thought of as being possible if the level of inhibition in vertical pathways is modified, and if the horizontal pathways are increased, in a “gated fashion”. Vertical pathways are thought to include thalamocortical fibers, as well as other input and output fibres. These findings are consistent with a notion that LTP induction is possible once a window of opportunity is opened, and thus the cerebello-thalamo-cortical pathway is hypothesized to play a role in gating the signals that allow for restructuring of the M1 output map.

Motor Adaptation, Learning, and M1: Studies

There is substantial evidence that shows the primary motor cortex changes in terms of physical map size and also in plasticity after learning a set of skilled motor actions. Studies in non-human primates have demonstrated that electrical stimulation maps of M1 change after learning a novel visually guided tracking movements and also in fine grasping tasks.80,105 Despite demonstrating that there is a change in the output maps of M1 post-training, there is a fundamental ambiguity in
the methods, since one cannot establish a baseline of performance for these motor actions until after they are learned; hence, there is no true way of gauging the improvement in performance—just that it was learnt. These studies, hence, have a limitation in that one cannot rule out that the acquisition of these adaptation abilities is due to altering a set of previously existing motor actions, and not learning a completely new one.

On the other hand, the stages of learning motor adaptation can be more definitively controlled, since it is possible to test the association of a neural response to an initially irrelevant, meaningless sensory cue and a pre-determined motor action: to determine a baseline and subsequently test the “learning” of a new association between them. Motor adaptation is an umbrella term referring generally to the association between a novel sensory cue and some sort of unrelated motor action. There are two types of motor adaptation: conditional association and sensorimotor adaptation. Conditional association refers to learning the relationship between a movement and a sensory cue that reports and determines the subsequent movement pattern. An example of conditional association is where Mitz et al. test subjects who manoeuvre a joystick in response to a visual cue that guided a cursor controlling the joystick. Sensorimotor adaptation, on the other hand, refers to establishing a relationship between an arbitrary visual cue and a well-learned movement. Wise et al (1998) studied this type of motor adaptation, and found that neurons were readily active in M1. Mitz et al on the other hand found more neural activity in the premotor cortex as opposed to M1 with a conditional association task.

Aside from indexing the association between M1 activity and a dedicated motor adaptation or learning task, another interesting approach is to study what happens to M1 with movement repetition and practice. It appears that motor representations in the human M1 are sensitive both to short and long-term experience, with changes been seen with as little as 5-10 minutes of repeating movements, though most studies report the more robust changes happening after 20-30 minutes of repetition. It also appears that prior practice on a task does not affect the subsequent changes in activity of the movement-related M1 representation. Karni et al (1995) studied repetition of either a frequently or infrequently practiced finger movement sequence using fMRI and found that repetition of the sequence, whether frequently or infrequently practiced, resulted in a similar decrease in M1 activation.
Despite these findings, there are many other studies that argue the opposite—that M1 activation increases with movement repetition\textsuperscript{34,109,110}, and these discrepancies are thought of as being due to differences in methodology (i.e. imaging techniques such as PET vs. MRI) and the behavioural measures used by these studies to study the resulting change in brain activation (i.e. using accuracy vs. reaction time). Nonetheless, it is for certain that these tasks modulate the resulting M1 activation. Recent work also puts forth the notion that M1 establishes functional connectivity with nearby related cortical regions, as demonstrated in Andres et al’s electroencephalography study that reported temporally correlated activations during early phases of learning co-ordinated manual sequences\textsuperscript{111}.

**Sequence-specific Learning: M1**

There are two general types of motor sequence learning studied: repetition of a sequence after observation, and un-cued, and repetition of a movement sequence cued by sensory stimuli. The role of M1 in these two types of sequence learning has been demonstrated in numerous studies. M1 activation changes after humans learn and practice a movement sequence in absence of sensory cues, such as tapping out a sequence. Changes in these tasks can happen even with minimal practice\textsuperscript{112,113}, though extensive practice may be needed to produce modifications in M1 representations beyond that which was observed prior to learning\textsuperscript{114}. There is also a segregation of fMRI increases in M1 movement-related activation, where experts of that particular movement sequence show increases in BOLD activity in M1, but non-experts show decreases, as evidenced in a piano playing task with musicians and non-musicians. From these studies by Pascual-Leone et al., it was concluded that M1 representations change due to repetition of a particular sequence of movements, but not when the movements are unrelated. This result suggests that M1 is involved in processing information regarding sequence-specific learning.

M1 demonstrates plasticity in relation to repetition of learned movement sequences performed from memory, but M1 representations also changes when humans learn sequences in response to sensory cues. This type of research methodology has predominantly been focused on establishing methods to parse implicit and explicit phases of learning, and a common example is the serial reaction time task (SRTT), where changes in reaction time occur in response to pressing buttons in a specific sequence when given a cue. Participants often had a remarkable decrease in reaction
time in this task without explicitly being aware of the sequence. This process has been deemed as acquiring implicit sequence knowledge. On the other hand, if subjects can recite the order of the sequence after the task, they have reached the declarative stage of learning where knowledge of the sequence has become explicit. This relatively simple task can assist scientists in assessing which brain regions are involved in the different phases of motor sequence learning. Pascual-Leone et al showed in an SRTT that transcranial magnetic stimulation (TMS) can activate larger areas of M1 representations of finger muscles during the implicit learning phase, and that the degree of change is proportioned to the reaction time decrease. Interestingly, once explicit knowledge occurred, M1 representations stopped expanding, and returned to baseline or went below baseline as participants continued to practice the sequence. Similar changes have been documented with EEG, where the alpha band during event-related desynchronization decreased during implicit learning, and showed a spike when sequence knowledge became explicit, then continued to decline.

Further evidence suggests that M1 may be involved with the effector rather than the actual learning aspects of visually-guided motor sequences. M1 activation changes are coupled to the effector muscle used in the task, and to changes in reaction time, suggesting a close relationship between the task and the subsequent motor actions formed. Secondly, if participants are informed of the sequence or the sequence order, the M1 activation no longer occurs during the SRTT. Based on this evidence, it would seem that the role of M1 in actual learning is not clear, since it appears to have a greater role in modulating the effector muscles, rather than tying in specifically to the implicit and explicit learning phases of the SRTT. Thus, more evidence is required to validate its direct participation in motor learning.

**Primary Motor Cortex and Plasticity with Learning**

One good question is to ask whether M1 is a substrate responsible for coding motor skills: if it is responsible, then functional reorganization will occur in this area after learning a motor skill. Rioult-Pedotti et al discovered direct evidence that skill learning permanently changes the motor cortex in rats during a skilled reaching task. There was an increased efficacy of layers II-III horizontal connections of M1 in slice preparations of these mice in the M1 contralateral to the
trained arm, but not in the M1 contralateral to the untrained arm. Field potentials evoked across layers II-III can be used as a measure to assess the efficacy of synaptic connections, and has been shown to be significantly and consistently larger in the trained M1 compared to untrained M1. This enhancement is linked to an LTP-like mechanism in plasticity, and after learning occurs, this LTP decreases, suggesting a learning-associated LTP. These studies investigated the impact of learning on the physical connections, but these paradigms have also been used in neuroimaging to explore the association of M1 horizontal connections activating during specific phases of learning. It remains unknown whether the functional reorganization of M1 occurs due to learning, or whether these changes influence the resulting learning, but these experiments directly provide evidence that learning a new motor skill changes M1 circuitry at a synaptic level. Thus, the primary motor cortex plays a critical role in motor learning, and is no longer regarded upon as a passive substrate. Next, we will discuss another neural region of interest: the prefrontal cortex.

Part 5: The Prefrontal Cortex

Executive functions, or the ability to manage and selectively modulate attention and decision making, is an ability unique to organisms with a neocortex. It is perplexing how specific neuronal arrangements and signaling can collectively allow an organism to carry out a wide variety of tasks, be it sensorimotor or cognitive. With the evolution of more complex organisms and an increased number of neurons, this introduces into the organism’s nervous system more capabilities and more neurons to allocate responsibilities to—yet, there is also more opportunity and room for confusion due to rich informational input. Thus, to curb confusion and most optimally allocate an organism’s energy towards what is important in its environment, it is necessary for a region of the brain to specialize in selectively modulating attention and decision making, to make survival and function optimal for higher organisms. The prefrontal cortex (PFC) is predominantly responsible for these executive functions, and in comparative neurobiology, it has been shown that humans have a much larger prefrontal cortex than other species. Using microelectrode recording in the monkey, Fuster et al. discovered large numbers of prefrontal cells that undergo altered firing throughout the delay between cue presentation and the
choice-making in a choice reaction time task. They concluded that there were two types of cells that responded in this type of task: one type responded particularly in the short-term memory process, and the second type responded during movement preparation. These cells were found mainly in certain areas of the dorsolateral prefrontal cortex\textsuperscript{121}.

The prefrontal cortex is an area of the cortex that plays important roles in working-memory, top-down modulation of sensory processing, cognitive control, and executive function\textsuperscript{122}. Histologically and physiologically, the cortex in the anterior pole of the frontal lobe can be divided into three major parts: the primary motor cortex, the premotor cortex, and the prefrontal cortex. The prefrontal cortex is the largest and most anterior of the three components\textsuperscript{121}, and is connected with many other cerebral structures, including: (1) reciprocal connections with the anterior and dorsal nuclei of the thalamus; (2) reciprocal connections with several sensory processing areas of the posterior association cortex; afferent inputs from various sensory systems also arrive at the PFC, and therefore PFC may be considered a cortex of multi-modal sensory convergence and association; (3) Reciprocal connections with limbic structures, such as the amygdala, hippocampus, and hypothalamus. Thus, the PFC is also implicated in aspects of emotion, motivation, and memory; (4) efferent connections to subcortical structures involved in motor control, such as the cerebellum, basal ganglia and the thalamus. Thus, the PFC has indirect connections to motor areas of the frontal lobe. Due to its widespread connections, the PFC is implicated in various processes, including emotion, learning, reward and decision-making. There have been attempts as well to compartmentalize the subdivisions of the PFC according to their functions, as well as assign lateralization to each hemisphere to define the PFC’s function. These will be discussed in the next section.

**The HERA Model and Functional Segregation of the PFC in Learning**

The HERA Model, initially proposed by Tulving, states two main hypotheses regarding the lateralized, specialized role of the prefrontal cortex: that on one hand, the left prefrontal cortex is predominantly more involved in retrieving information from semantic memory and in encoding of episodic memory, compared to the right prefrontal cortex. On the other hand, the right prefrontal cortex is preferentially more involved in retrieving episodic memory than the left\textsuperscript{123,124}. Episodic memory refers to the ability to remember information in its spatio-temporal
context, as defined by Tulving\textsuperscript{125}. Encoding is the ability to generate a memory trace with new information, while retrieval is a process allowing for conscious experience of remembering a past event. There have been many studies done, many of which support this notion that there is a hemispheric specialization of the PFC, regardless of whether the material that is being processed is verbal or non-verbal\textsuperscript{126}. On the other hand, some studies support that the PFC asymmetry depends on what kind of material is being processed\textsuperscript{127–129}. In these studies, authors suggest that the left PFC is more involved in verbal processing whereas the right PFC is preferentially activated in non-verbal material processing.

However, the potential problem with the HERA model is that it assumes that the entirety of the PFC assumes the same function, when in reality there are sub-regions of the PFC that may take part in very different roles during encoding and retrieval of episodic memory. For example, the ventrolateral PFC (VLPFC) is activated when encoding successfully occurs\textsuperscript{130}, whereas the DLPFC is activated more when organizational strategies are required during encoding\textsuperscript{131–133}. Despite this assumption, the HERA model provided important insights and sparked an inquisition into the functions of the various sub-compartments of the prefrontal cortex. One particularly useful method is through studying patients with lesions in the prefrontal cortex.

**Lesional Patient Studies: Uncovering the Prefrontal Cortex Function**

An indirect approach to study the function of the PFC in learning is to observe the effects of lesion in that area to the subsequent impairments in function. Researchers have documented that lesions to the DLPFC can result in a wide range of cognitive and executive deficits, including working memory, rule learning, planning, attention, and motivation\textsuperscript{134}.

It was shown in numerous studies\textsuperscript{135–139} that damage to the PFC causes memory deficits only discernable in the most complex memory tasks, such as patients being impaired when asked to freely recall items or patients reducing their use of organizational strategies in memory tests\textsuperscript{135}. The results from studies that segregate damage to left and right PFC to observe the effects of this damage on semantic encoding were trivial, since the impairments returned to normal levels when categorical cues are provided continuously during encoding and retrieval\textsuperscript{140,141}. Additional studies provided inconclusive results regarding the effect of the lesion site having a specific effect of impairing memory encoding and retrieval, and a possible reason for these discrepancies
may be because, again, the studies were not specific enough to sub-compartmentalize PFC. The ultimate reason behind these variations could be due to the fact that these studies focused on studying PFC lesions as a whole, and did not attempt to organize patient lesions by their specific locations. It is possible that different subareas of the PFC are engaged in varying stages of the memory acquisition and retrieval process.

Koch et al (2006) studied patients with medial or lateral prefrontal lesions. Their findings in summing the reaction times of all patients support the notion that prefrontal cortex lesions do not impair sequential learning, neither do they impair the “chunking” strategies used during sequence learning, as evidenced in reaction times during the serial reaction time task\textsuperscript{142}. However, pooling data from all patients with PFC lesions may be misleading since it is clearly described in this paper that different groups of patients had lesions in different aspects of the prefrontal cortex (medial vs. dorsal), and these two areas may have different implicated functions in learning. From the data given of segregated lesion patient performance, the patients with medial PFC lesions (N=18) seemed to perform worst in the SRTT compared to controls, and those with lateral PFC lesions (N=16) seemed to perform closest to the level of controls. Another problematic aspect of assuming that lesions in either medial or dorsal PFC does or does not affect learning in this study is that when grouping subjects, there was no distinction between left and right lesions, meaning that it is assumed that the left and right subareas of the PFC perform similar functions, when in reality there is not enough evidence to assume this is so.

Patients with lateral PFC damage also show impairments in free recall, such as when asked to recall freely learned word lists, while cueing these patients significantly improves their recall performance\textsuperscript{135,143,144}. These deficits can occur both during retrieval and encoding stages of learning\textsuperscript{135}. In addition to these impairments in free recall, lateral PFC patients also show impairments in the temporal ordering of events, which signifies that there may be impairments in these patients pertaining to strategic retrieval\textsuperscript{136,145,146}. The results from these studies suggest that patients with lateral PFC damage, especially to the DLPFC, are not able to organize information that they previously learned effectively to facilitate their recall. Shimamura et al (1995) proposed that the impairments observed in these patients results from a failure of the PFC to either inhibit noise, or unwanted information, or select among competing memories. This causes more recently active memories to interfere with the ability to retrieve more distant ones learned in the
past\textsuperscript{145,147}, providing evidence for the strikingly important role of the DLPFC in temporal ordering of events during encoding/recall, and in action selection.

Aside from impairments in recall after lateral PFC damage, there are less common reports of impairments in recognition. More specifically, the DLPFC lesioned patients revealed deficits in familiarity-based recognition only when the lesioned hemisphere is involved in the encoding process\textsuperscript{139,148,149}.

In addition to these ambiguous results, there is an inherent limitation to interpretations in lesions research, because in patients who have experienced lesions, there may be other processes at play that attempt to counteract the damages set forth by the lesion. The brain is remarkable in that it is plastic, and these compensatory mechanisms and other neural processes can therefore obscure the effect of the lesion on the subsequent cognition and performance\textsuperscript{150}. Therefore, it is important to consider other methodological approaches to study how disabling a particular brain area has an effect on the resulting behaviour. One non-invasive approach to address this is to use transcranial magnetic stimulation, which applies magnetic pulses against the skull to excite the underlying neurons. Applying repeated pulse of TMS at a specific frequency can cause a “virtual lesion” in a cortical area by temporarily inhibiting function of those local neurons\textsuperscript{151}. Moreover, TMS exudes its inhibitory effects by decreasing the excitability of the cortex at low frequencies on the healthy human brain\textsuperscript{151,152}. Therefore, a methodological approach using TMS or other non-invasive brain stimulation can potentially help in examining and segregating the functions of the left and right DLPFC in particular cognitive functions.

**Brain stimulation studies of the DLPFC and the Dopaminergic Pathways**

In this section, we will discuss evidence from studies that dopaminergic neurotransmission in the striatum can be facilitated by high-frequency rTMS of the DLPFC, and also studies that show that rTMS of the DLPFC can cause observable and quantifiable changes in learning and behaviour. We will also discuss possible implications of the DLPFC in motor learning.

The DLPFC is part of the mesocortical dopaminergic pathway that connects the ventral tegmentum to the cerebral cortex, in particular, the frontal lobes. The mesocortical pathway is essential to normal cognitive function and operations of the DLPFC. One of the four major
dopamine pathways is the nigrostriatal pathway, which connects the substantia nigra with the striatum. This particular pathway is involved with the production of movement, and is a part of the basal ganglia motor loop.

In the following paragraphs, we will explore some of the major studies that focused on this particular topic of rTMS on the DLPFC.

Studies on the role of the DLPFC in working memory provide a body of evidence that supports its role in certain aspects of this cognitive domain. Neuroimaging studies have found that rTMS alters neural activated in the stimulated area as well as in remotely connected area. For example, PET studies found that high-frequency rTMS over the prefrontal cortex can modulate subcortical neurotransmission\textsuperscript{153–158}. In particular, Strafella et al. used 10 Hz rTMS to the left prefrontal cortex of healthy subjects and used radiolabeled ligand C\textsuperscript{11} raclopride to assess dopaminergic receptor availability. They found that compared to a control stimulation site, high-frequency TMS over the left DLPFC increased endogenous dopamine release in the striatal region. Likewise, similar results have been discovered in patients with Parkinson’s disease and depression\textsuperscript{154}.

There is substantial evidence that the DLPFC is engaged in encoding and retrieval of episodic memory\textsuperscript{130,132,159,160}. There is also evidence that indicates a lateralization of organization of working memory functions between the left and right DLPFC. Despite their undoubted involvement in working memory, the challenge for researchers currently is to determine and segregate the functional responsibilities of the left and right DLPFC, and whether or not the these brain areas are compartmentalized by (1) the type of information being encoded (verbal vs. non-verbal information) or (2) the stages of acquiring a new memory (whether left vs. right are responsible for solely encoding or retrieval of episodic memory traces).

Neuroimaging studies have strengthened the idea of hemispheric asymmetry for memory processing, regardless of the type of information being learned (verbal vs. non-verbal). On the other hand, there are studies that indicate that the left vs. right DLPFC asymmetry may depend, to a certain degree, on the type of material. An example of a study where evidence suggests that the function of DLPFC is lateralized according to the type of information being learned is where Sandrini et al.\textsuperscript{161} used facilitative (10 Hz) repetitive transcranial magnetic stimulation (rTMS) to
left and right DLPFC on subjects who partook in the n-back task, which is a test of working memory, where subjects are presented sequentially with letters and tested whether or not a particular letter matched the letter displayed n-trials previously. To succeed in the n-back task, subjects must not only monitor, update, and manipulate information, but they must also be able to suppress task-irrelevant information. Sandrini et al found that rTMS affected subject performance of the n-back task depending on the type of information being provided. In these tasks, subjects were required to focus on only one specific element of the letter (either location or letter), but they were also simultaneously presented with distractor elements that were irrelevant to the task (either verbal or spatial). Sandrini et al. concluded that because the rTMS of the left DLPFC disrupted performance in the location task when verbal distractors were present, the left DLPFC is selectively responsible for encoding location-specific information. On the other hand, rTMS of the right DLPFC in the letter task disrupted performance in the presence of spatial distractor elements, suggesting that the right DLPFC is important for suppressing spatial distractors to encode the relevant information in the task. The results from this study support the idea that the PFC is involved to coordinate executive aspects of the task, that functions of the DLPFC are lateralized. Evidence supports that the PFC is responsible for top-down modulation and control of attention processes, providing evidence for the theory that working memory controls selective attention through a top-down process in the human brain. Previous neuroimaging studies are in agreement with the present interpretation, where Smith & Jonides demonstrated that PFC is markedly organized according to the type of material or information: the left PFC for verbal information, and the right PFC for non-verbal information.

Mottaghy et al. studied healthy subjects by applying 4 Hz rTMS to either the right or left DLPFC and observing the subsequent effects on working memory via the 2-back verbal working memory task, compared to rTMS of the vertex. They found that stimulating both right or left DLPFC worsened the subsequent working memory performance in the working memory task, and PET imaging also showed significant reductions of rCBF at the stimulation site, and in interconnected brain regions—this suggests that rTMS can have a temporary “lesion-like” effect and functionally impair cognitive aspects of functioning, and particularly working memory, when the DLPFC blood flow is disrupted. Meanwhile, Javadi et al. used transcranial direct current stimulation (tDCS) of the DLPFC. This study showed that anodal tDCS improved declarative verbal memory compared to sham when stimulation occurred in encoding phase, wheras
cathodal tDCS impaired memory performance when applied in both encoding and later recognition. tDCS on M1 had no such effect: Javadi’s study suggests that the DLPFC plays a site-specific role in declarative verbal memory. Many further studies have confirmed and elaborated upon evidence that anodal tDCS of the DLPFC can modulate and enhance the resultant working memory, in both healthy subjects and in patients with stroke and Parkinson’s disease\(^{166-170}\).

However, aside from the abundance of evidence suggesting the DLPFC’s role in working memory, there is also evidence that DLPFC does not play a direct role in storage of working memory during learning tasks. Postle et al.\(^{171}\) provided evidence that the DLPFC is responsible for control of information in working memory, as opposed to its short term information retention, through a TMS-fMRI study in two conditions of a working memory task: one where subjects were required to remember certain letters of the alphabet (maintenance condition) and another condition where subjects are required to reorder the set of letters provided to them alphabetically (manipulation condition). Results indicated that rTMS of DLPFC the ability to manipulate and maintain provided information, disruption of the superior parietal lobe disrupts performance to the same extent. Their findings are consistent with the notion that the DLPFC is not uniquely involved in short-term retention domain of the working memory process, but rather perhaps contributes in other aspects.

**DLPFC: Motor Control & Preparation**

The combination of non-invasive brain stimulation studies show that modulating and stimulating the left DLPFC has facilitatory effect on working memory, but few studies have been done to explore the functional regional circuitry that DLPFC is involved in.

Within this prefrontal cortex, the dorsolateral prefrontal cortex (DLPFC) plays specialized and crucial roles for motor control and behaviour. The DLPFC is referred to as a functional area, but the Brodmann’s areas 9 & 46 (BA9 & BA46) are neuroanatomical areas of the cortex that largely overlaps this, and hence roughly corresponds to the DLPFC. These areas have various connections of neurons that extend to several motor regions in the basal ganglia, cerebellum, supplementary motor area, and premotor cortices, as evidenced from studies in monkeys\(^{172-174}\). It is worthwhile to note at this point that while there are no direct anatomical connections between
BA9/BA46 and the primary motor cortex, though there has been research that demonstrates functional connectivity between these two regions during procedural motor learning, at a sub-second timescale\textsuperscript{175}. This is in contrast to a previous study by Terao et al\textsuperscript{176}, who found negative results of DLPFC’s role in motor preparation. This group applied inhibitory 1 Hz rTMS to examine the subsequent RT delay in a go-signal button pressing task. Subjects were required to press one of two buttons, after a cue displays which hand and which button to press. A pre-cue was presented before the cue that either conveyed advance information about the cue, partial information, or no information. rTMS was applied after baseline measures in the RT were obtained, and RT measures were repeatedly taken at set time intervals after the rTMS. DLPFC had minimal change in delay, regardless of the type of pre-cue presented, whereas other areas like PMC and M1 and SMA had significant delays. This negative finding suggests that the DLPFC is not critically involved in cortical processing for motor preparation. However, this study only examined the resulting changes in the behavioural measures after rTMS, as it was designed in an interventional paradigm. More studies are needed to confirm the role of DLPFC on the resulting motor output in a more temporally-associated format.

**The role of the DLPFC in Motor Sequence Learning: Implicit or Explicit**

The role of DLPFC in motor learning is complex and has been debated for a long time. There has been evidence for involvement of the DLPFC in both explicit and implicit learning.

Ghilardi and colleagues, using a sequential spatial reaching task, found significantly larger PET activations in the left DLPFC when subjects were required to discover the correct order of a repeating sequence by trial and error, and also when they were instructed to just remain immobile and attend to the same task without actually reaching with their hands, compared to when subjects had previously been given a sequence and had practiced this known sequence\textsuperscript{23}. This suggests that regional, left DLPFC activation is associated with processes related to pattern or sequence searching through observing spatial cues, despite not knowing the sequence ahead of time. Furthermore, neuroimaging studies have shown that the DLPFC is activated during the fast, early learning stages\textsuperscript{11,109}, even when subjects did not have prior practice or knowledge of the sequence, and that this activation was specific to sequence-learning and not to non-specific
learning\textsuperscript{177}. This is also in agreement with other studies, which also reported the involvement of DLPFC and activation during learning of new motor sequences\textsuperscript{19}. It has been postulated that the DLPFC may be involved in either encoding or acquisition of the eventual explicit knowledge of the task\textsuperscript{45}.

The activity in prefrontal cortex remains high when sequence-specific learning occurs\textsuperscript{11,19–21,26,45,178–180}. Even more convincing evidence of sequence specific learning in non-human primates have been shown in recording sequence-specific cells of the lateral PFC during production of a spatially-cued sequence.\textsuperscript{181} However, in humans, when reproducing a well-learnt sequence from memory, similar cell recording studies showed no change in activity\textsuperscript{19,182–184}; rather, the production of sequence-related movements was related to increased SMA activity. The evidence from these studies point towards a dissociation in function between the prefrontal cortex and the SMA. Therefore, there is strong evidence that the PFC is necessary early in learning, when the subject sequence representation in insufficient to drive behaviour without the externally-cued stimuli that indicate the appropriate responses to the subjects\textsuperscript{21}.

Pascual-Leone et al (1998)\textsuperscript{185} further supported this notion of the PFC’s role in early sequence learning by discovering evidence that supports dissociation between DLPFC and SMA during sequence learning using TMS. TMS was applied continuously during SRT training, and the coil was placed over top the SMA, ipsilateral DLPFC (to the training hand), or the contralateral DLPFC, or a control group that did not receive magnetic stimulation. TMS did not affect the reaction times for the subjects in each group; however, compared to the control condition, TMS over the contralateral DLPFC significantly reduced sequence learning. This study showed that the effect was site-specific: no effect was seen with stimulation of SMA or ipsilateral DLPFC.

Shadmerh & Holcomb showed with PET that during a sequence motor learning task, prefrontal areas were active during the early stages of practice in the sequence condition compared to a control condition, and this activation does not change much between the early and late learning conditions. This suggests that improvement in learning-specific performance (in sequence vs. RANDOM conditions) was at least in part due to the activation of the visuomotor association areas in the prefrontal cortex. The activations eventually shift to the premotor, posterior parietal, and cerebellar cortex structures 6 hours after practice was completed\textsuperscript{34}. 
However, there is a body of evidence that also supports a role for the DLPFC in explicit learning. A continuous theta burst TMS study in motor learning by Wilkinson et al\textsuperscript{186}, showed that applying excitatory cTBS to M1 had improved motor learning during its early stages, while stimulating the DLPFC with inhibitory TBS had improved learning in the later stages of the task in a modified probabilistic serial reaction time task. This evidence suggests that inhibition of the DLPFC has an effect on explicit motor learning, and that perhaps the DLPFC plays a role in explicit learning. This study was in agreement with a study by Nitsche et al (2003)\textsuperscript{187} using anodal tDCS to suggest crucial involvement of M1 in explicit sequence learning.

**DLPFC and M1 Connectivity**

There have also been studies with TMS applied during a task to evaluate how stimulating the DLPFC affects the resulting on-line performance. Hasan et al.\textsuperscript{175} used dual site TMS to explore how stimulating the DLPFC, or Brodmann area 46 in this case, at specific intervals, both (1) stimulus onset asynchrony (SOA), defined as the interval between onset of the cue and onset of brain stimulation, and (2) interval between the conditioning stimulus on DLPFC and the test stimulus on M1. The task used was a free or specified choice selection task. This study found that a DLPFC conditioning pulse delivered 100ms after cue significantly enhanced excitability of M1 for the free choice trials, with the optimal interstimulus interval (ISI) between DLPFC and M1 being at 12ms. At 75 ms SOA, the opposite effect was seen, where the excitability of M1 was enhanced more for externally specified cue trials than for free choice trials. These significant findings were specific to timing and also to the index finger muscle involved in the button pressing during the task. Furthermore, stimulating an area adjacent to BA46, namely BA9, did not show a similar effect, suggesting a site-specific effect. Therefore, these findings suggest that there is timing-specific and muscle-specific connectivity between DLPFC and M1, and that this association is important to study in order to further delineate the DLPFC’s role in motor learning.
CHAPTER 2: RESEARCH AIMS AND HYPOTHESIS

RESEARCH AIDS

The purpose of this research study is to determine the role of the dorsolateral prefrontal cortex to M1 connectivity during motor sequence acquisition and how it changes with aging. Despite knowing that the DLPFC is a critical component involved in motor learning, there is very little insight into the functional connectivity of this area to M1, and whether this connection changes with age.

HYPOTHESES:

Age is associated with changes in connectivity and integrity of the brain structure. Thus, we hypothesize that there is a correlation between age and DLPFC-M1 functional connectivity, and that aging will decrease this functional connectivity in older, healthy subjects during motor sequence acquisition. Thus, the changes in DLPFC-conditioned M1 MEP with motor sequence training will be less in older subjects compared to younger subjects.

Motor sequence acquisition is associated with changes in DLPFC-M1 functional connectivity, and we will see an initial facilitation in DLPFC-conditioned M1 MEP in early sequence training (between PRE and DURING), but not random training. This facilitation will decrease with further motor sequence training (from DURING to POST 0 and POST 30). Since previous neuroimaging studies have shown that BOLD activity in the DLPFC transiently increases, and then subsequently decreases with motor sequence training, we hypothesize that DLPFC is involved with active maintenance of attention to spatial cues early in sequence acquisition, but this process becomes less active with training, as subjects learn the sequence. Thus, there will be a decrease in DLPFC-M1 functional connectivity through the gradual process of acquiring a new visuomotor spatial sequence.

We also hypothesize that these changes in DLPFC-M1 functional connectivity are correlated with changes in the behavioural outcome measures (reaction time and skill measures that we model in the study).
CHAPTER 3: MATERIALS & METHODS

3.1: Subject Recruitment/Study Design

All subjects were recruited through poster advertisements placed across various teaching hospitals and community centers across downtown Toronto, and were screened by telephone to ensure eligibility for the study. Subjects who passed the screening test reported that they were right-handed and had:

- No history of any neurological diseases or disorders
- No previous history of chronic shaking or tremor in any body part
- No previous history of head, neck, or spine trauma
- No history of psychiatric or psychological disorders
- No previous history of stroke or seizure
- No metal objects implanted in the body
- No previous muscle weakness/tingling/numbness in the hands or wrists
- No diagnosis of carpal tunnel syndrome
- No loss of hearing or ringing in the ears
- Not been taking any medications that would affect the central nervous system

Study Design

14 right-handed healthy subjects cleared the telephone screening and were deemed eligible for this research study. All subjects provided written informed consent in accordance with the Declaration of Helsinki. This research protocol is approved by the University Health Network (Toronto) Research Ethics Board.

Each of the subjects participated in two 3-hour visits arranged at least three days apart to minimize carry-over effects. These two visits were counterbalanced and identical except for the
motor sequence acquisition task: in one visit the task contained a sequence (SEQ) and in the other visit it did not contain a sequence (RANDOM) (see Behavioural Task section for further details). A subgroup of these subjects (N=10) participated in a third visit where they had an MRI anatomical scan completed, for the purpose of validating our stimulation sites (see MRI visit section below).

At their first visit, subjects who were naïve to transcranial magnetic stimulation were assessed by a neurologist to ensure that they were healthy and that there were no signs of neurological disorders. Those who had previously participated in a TMS study in our laboratory bypassed this step, as they already had a neurological examination previously. After subjects cleared the neurological examination, their handedness was re-assessed using the Edinburgh Handedness Inventory to ensure that they were right-hand dominant.188

For each of the two task-related visits, TMS measures were taken at baseline (PRE). Next, the subjects engaged in the Serial Visual Isometric Pinch Task (described in subsequent section), which will be abbreviated as the “pinch” training task, and TMS measures were re-taken at various time points with reference to the pinch task: after two blocks of training (DURING), immediately after training (POST 0), and thirty minutes post-training (POST 30). During the sequence visit, subjects were asked immediately after their training (before POST 0 TMS) if they recalled a repeating pattern during the task, and if they could recall a pattern, we recorded their self-reported pattern sequence to gauge accuracy of what they remembered.

Finally, if subjects had participated in an MRI scan, the Brainsight neuronavigation system was used after the experiment to localize the stimulation site of the left DLPFC, as defined by MNI co-ordinates in previous neuroimaging studies, and the left DLPFC defined as the scalp-marked site relative to the primary motor cortex hotspot as well. The Behavioural Task, TMS, MRI, and Brainsight sections following this will explain in more detail each of these components of the experiment. The schematic below summarizes the timeline of visits for a typical subject:
Figure 3: Schematic detailing the experimental visits for each subject, broken down into individual components. Subjects participated in a total of 2 experimental visits, and a potential separate MRI visit (not shown), if applicable. Subjects engaged in a motor pinch training task, and TMS measures were obtained at baseline, after 2 blocks of training, immediately after training, and 30 minutes after training. After training in the sequence task, subjects were asked if
they recalled any repeating pattern. After the TMS experiment stimulation location of the DLPFC was then recorded with Brainsight.

3.2: Electrophysiological Recordings

Electromyographic recordings (EMG) were obtained through surface electromyograms from the first dorsal interosseous (FDI) muscles of each subject with 9 mm diameter Ag-AgCl surface electrodes. The active electrode was placed on top of the muscle belly, and the reference electrode over the metacarpophalangeal joint of the index finger. Ground electrodes were placed around the wrist and also on the back of the hand to minimize interference. The electrical signal was amplified (1000x), band-pass filtered (20 Hz – 2.5 kHz, Intronix Technologies Model 2024F), digitized at 5 kHz by an analog-to-digital interface (Micro1401, Cambridge Electronics Design, Cambridge, UK) and stored in a secured computer for off-line analysis.

Behavioural Measures: SVIPT (Serial Visual Isometric Pinch Task)

Apparatus set-up:

A 5-kg load force transducer [Transducer Techniques, LSP-5] was connected to a digital-to-analog data acquisition device [National Instruments, USB-3000], which was connected to a laptop [MSI, model #11230BNHMW]. A Labview program was designed on this laptop (Labview Version 13) and the task featured 5 horizontal, rectangular bars arranged to be equidistant from each other in the center of the screen, from top to bottom (see Figure 4). The bar at the very bottom, in yellow, was the “HOME” position that the calibrated cursor, at rest, would reside. During the task, the program sampled the cursor position at a rate of 50 Hz, and was designed to have a linear relationship with the force input sensed by the load transducer (i.e. more force input corresponded to a linear degree of change upwards in the cursor position, and the cursor can only move upwards or downwards).
Figure 4: Serial Visual Isometric Pinch Task. After calibration, cursor (red square) is at the HOME position (yellow bar) when the right hand is relaxed. When a target flashes (green bar), subjects were asked to squeeze the transducer to manoeuvre their cursor to reach the target and return to HOME as quickly and accurately as possible. (RT=response initiation time)

Calibration:

Subjects were seated approximately 50 cm in front of the computer monitor and were asked to complete a motor training task. Prior to the actual task, a calibration procedure was conducted to ensure that the cursor was in the HOME position when the subject was holding the transducer in a relaxed, resting position with their right hand. The subject was given an instruction to squeeze to a maximum force exerted by their right hand that could be sustained for 10 seconds or more, on a force sensor. This force value was then multiplied by 0.2 to obtain 20% of the maximal force, which was inputted into the program. The program was designed to calibrate itself such that, to reach the furthest target on the screen from the HOME position, the subject had to squeeze to 20% of their unique maximal force value. This value was chosen to prevent subjects from experiencing muscle fatigue by the end of the training.
**Pinch training task:**

When subjects engaged in the task, one of the four bars above the HOME bar would flash green 300 ms after the task began, and subjects were all given the same instructions to “squeeze the force transducer with their right hand to reach the green target and release the transducer to return to the HOME position as quickly and as accurately as possible. If the cursor touched the target, it would count as a ‘hit’; if it surpassed or fell short of the target, it would count as a ‘miss’. After the cursor returned to the HOME position in a trial, the next trial would begin right away”.

![Diagram of the pinch training task](image)

**Figure 5: Time course on a trial by trial basis.** A target cue is presented to the subject after 300 ms of the trial elapsed, and the subject is instructed to reach the target and return HOME as quickly and as accurately as possible. Outcome measures obtained in this task are response initiation time (RT), Movement Time (MT), and accuracy (binary and spatial). See Data analysis and statistics for more details on how each measure was obtained.

Subjects participated in two visits with different tasks. For the purpose of clarity the two visits will be termed SEQ and RANDOM. These visits were order randomized to ensure that there was no effect of visit order on the outcome measures. **Subjects were not informed that there would be a sequence in their task for both visits.**

For the SEQ visit, the ordering of the targets was in a 12-item sequence (1-3-2-3-2-3-4-1-2-2-4), and this sequence repeated itself 10 times for every block of training, for a total of 120 trials per block, and for 10 blocks of training in total (with the exception of block 9, in which the ordering of the targets was completely random for the 120 trials).
For the RANDOM visit, the ordering of the targets for the 120 trials was completely random for all ten blocks, generated from the “RANDOM” function on Microsoft Excel 2007. Each new block was different than the other blocks (unique random order). Refer back to Figure 3 for the schematic detailing the two visits: a green bar represents a sequence block, and a red bar represents a block with RANDOM (random order).

At the end of the 10 blocks of the pinch training for the SEQ visit, subjects were asked whether they noticed a repeating pattern during the task, and this recall was noted. If subjects reported seeing a pattern, they were asked to regenerate the sequence. The reported sequence was noted to assess the accuracy of recall. However, we did not assess recognition of the sequence (i.e. through giving a short multiple choice test with the actual sequence as one of the choices).

It is important to note here that our cross-over study design only assesses the acquisition of a sequence, but does not necessarily mean that motor skill learning actually occurred. Motor skill learning refers to permanent improvements in a given motor skill, and this is generally reflected in brain circuitry that has modified due to the skill acquisition. The motor skills acquired should be sustained, stable, and adaptable to new situations. In future sections, we will discuss characterizing motor skill in our pinch task, but this does not imply that learning has occurred.

**Transcranial Magnetic Stimulation**

TMS was applied to the left primary motor cortex and left dorsolateral prefrontal cortex with figure-of-eight shaped coils connected to Magstim stimulators (Magstim 200² and Magstim Innovation 4-into-1 combining module). The M1 hotspot for the right first dorsal interosseus (FDI) muscle was determined by first marking the scalp measurements of half the distance from the left to right tragus of the ear, and half the distance from nasion to inion. The location where both measurement trajectories intersect is labelled as Cz, as per the distances cited in the EEG 10-20 system²⁵⁰. C3 is then marked as 20% of the total intertragal distance to the left of Cz, and the starting point for finding the M1 hotspot is 1 cm behind C3. By varying coil location, the M1 FDI hotspot was determined by finding the location that yielded the largest output MEP in the target muscle.
Several measures were taken: resting motor threshold, single-pulse stimulation of M1, paired-pulse stimulation, and dual-site stimulation. For the coils placed on the M1 hotspot, the handles of these coils pointed backwards at 30 – 45° from the mid-sagittal line, approximately perpendicular to the central sulcus (this will be termed the “conventional orientation”). Pulses that are monophasic induce an electric field with a clear-cut peak that creates posterior-to-anterior directed currents in the brain. This current direction is known to activate pyramidal neurons transsynaptically, and evokes multiple descending volleys, termed indirect waves, in the spinal cord\textsuperscript{55}. For all TMS recordings, the TMS pulses were programmed to be delivered 6 seconds apart.

**Resting motor threshold (RMT)**

The RMT of each subject was taken after the M1 hotspot was localized in the left hemisphere, defined as the minimum stimulation output that would generate a motor-evoked potential (MEP) greater than 50 μV\textsuperscript{251}, in at least 5 of 10 delivered pulses when the FDI muscle is relaxed. This surface EMG signal was amplified 10,000x and band-pass filtered at 20 Hz – 2.5 kHz. When a stimulation output satisfied this criterion, this output value was recorded as the RMT and used to calculate the stimulation intensity for conditioning pulses in later recordings.

**Recordings for 1mV MEP**

At baseline recordings, a 70 mm coil (Magstim) was used to deliver ten TMS pulses (equivalent to 1 minute) to the M1 hotspot for the right FDI muscle in the conventional orientation, at a stimulation intensity that generated an average of 1 mV MEP, measured by calculating the peak-to-peak amplitude of the waveform recorded in the muscle. Ten TMS pulses at this intensity were delivered at different stages of the motor training task and the average of these pulses was obtained as a measure of the overall excitability of the primary motor cortex during the different stages of training (i.e. DURING, POST 0, POST 30).

**Paired Pulse Recordings.**

The same 70 mm coil in conventional orientation was also used for paired pulse recordings. The test stimulus is defined as the pulse delivered at an intensity that approximately generates an average FDI MEP size of 1 mV from peak-to-peak. The pulse preceding the test pulse is termed
the conditioning stimulus, and was set at 80% of RMT. The interval between the conditioning and subsequent test stimulus is the interstimulus interval (ISI) (see Figure 6 A and C for paired pulse TMS coil placement and EMG). The conditions tested were: test pulse only, ISI=2 ms, ISI=3 ms, ISI=10 ms, and ISI=15 ms. Ten trials for each condition were delivered in random order for a total of 40 trials at baseline, which required 4 minutes. At subsequent TMS testing time points (i.e. DURING, POST 0, POST 30), the test pulse was re-assessed, and adjusted if necessary to achieve an average MEP of 1 mV, and the 40 trials were repeated.

**Dual Site Recordings.**

Two coils were used for the purpose of these recordings: a 50 mm (test) coil and a 40 mm (conditioning) coil. The respective hotspots for the M1 hand motor area (FDI muscle) were determined again (in conventional orientation) and marked for these two coils, since the center of these coils are not the same as for the 70mm coil previously used. The RMT value for the 40 mm conditioning coil was also determined. Another scalp marking was made 5 cm anterior to the 40 mm coil M1 FDI hotspot, and this would be where the center of the 40 mm coil would deliver conditioning stimuli over the left DLPFC. The orientation of the DLPFC 40 mm coil was from anterior-to-posterior direction (coil wiring facing forward).

Prior to the actual recordings, the two coils were set up on the left hemisphere: the 50 mm test coil was placed in the conventional orientation over its M1 FDI hotspot, and the 40 mm conditioning coil was placed in an anterior to posterior orientation, with the center of this coil over the marking made for the left DLPFC. The stimulation output value for the test coil is determined as the value that elicits an average FDI MEP of 1 mV, with both coils still placed on their marked locations during this assessment.

Two sets of recordings, containing 40 trials each, were taken (which required 4 minutes of recording time for each), with the difference of each set being the intensity of the conditioning coil. Based on pilot data from our lab studying DLPFC-M1 TMS at various intensities and ISI’s, and also based on previously published studies of DLPFC-M1 TMS\(^{239}\), the stimulation output for the conditioning coil over the left DLPFC was 90% of the RMT for the first set of recordings, and then 110% of RMT for the second set of recordings. The recordings consisted of 40 pulses that cycle randomly across five possible conditions: test pulse only, ISI=4 ms, ISI=6 ms, ISI=8
ms, ISI=10 ms. For each ISI, the 40 mm coil providing the conditioning stimulus (left DLPFC) always precedes the 50 mm coil providing the test stimulus (left M1) (see Figure 6 B for coil placements for the conditioning and test coils during the dual site recordings, and Figure 6 C for the EMG recording output). At each subsequent TMS time point (DURING, POST 0, POST 30), the test coil stimulus is reassessed and the recordings of 40 pulses are repeated.
Figure 6: Figure A above shows the coil positioning for single and paired pulse TMS stimulation on the left M1 (posterior-to-anterior orientation, 45 degrees clockwise). Figure B shows the coil positioning of the 40 and 50 mm coils to stimulate the left DLPFC (anterior-to-posterior) and left M1. Figure C shows a sample output of a single motor-evoked potential from the first dorsal interosseus muscle, and also the paradigm for paired pulse stimulation (conditioning stimulus followed by test stimulus). Figure D shows the electrode positioning for the electromyography recordings: the red circle indicates the recording electrode on the active FDI muscle; black circle with “G” and black rectangle indicate the ground electrodes used to filter out noise; black circle with “R” indicates the reference electrode.
3.3: Magnetic Resonance Imaging (MRI) Visit

10 of the 14 subjects attended an MRI session on a separate date, wherein anatomical imaging was conducted to locate and subsequently mark which brain areas were stimulated using TMS. Subjects arrived roughly one hour prior to their scan time to allow for adequate preparation time. TMS and EMG recordings were used to determine the primary motor cortex hotspot for the hand (i.e., the area on the left hemisphere where an applied TMS pulse would yield the largest contraction of the right FDI muscle at rest). Next, the location of the left dorsolateral prefrontal cortex was marked 5 centimetres from the M1 FDI hotspot, as this has been previously determined to be a reliable method to locate this area.\textsuperscript{240,241} Electroencephalography (EEG) caps were then tightly bound to the head of the subject, and vitamin E capsules were taped securely on top of where the marked left M1 and left DLPFC were localized on the head. The purpose of the capsules is to visualize on the scan the exact area of the head where the markings were made, which will assist in determining where on the brain these markings correspond to.

Some, but not all (10/14) participants underwent an anatomical MRI on a 3.0 T MR-imager (GE; surface coil designed for high SNR and 500 min-plane resolution; gradient-echo spiral EPI) at the Toronto Western Hospital. In each participant, an individual T1-weighted anatomical three-dimensional MR-image set (172 slices, slice thickness=1.1 mm) was acquired and the process took about 15 minutes. The MRI was used to reconstruct a three-dimensional brain using BrainProject (Rogue Research Inc.), and neuronavigation was used to localize the DLPFC after TMS.

Neuronavigation and Validation of Stimulation Sites (BrainSight)

A three-dimensional model brain of each subject who took part in the MRI scans was created through a brain reconstruction process, where the slices taken from the scan are compiled together. First, images from the anatomical scan for each subject were uploaded onto the iMac Computer (Apple, Inc.). Next, using BrainProject, the slices were used to create a new project, and using a curvilinear tool, the curvature of the brain was traced in the axial, coronal, and sagittal views by hand (roughly corresponding to the dura surrounding the brain). The final three-dimensional brain was then verified to have been appropriately traced, and specific landmarks were programmed into the project that were deemed to be reliable landmarks to use when
calibrating subjects (bridge of nose, tip of nose, nasion, inion, intertragal notches of left and right ears). This was then registered into the Brainsight program, where validation and brain marking could take place after subjects complete the TMS experiment.

Validation of Stimulation Sites

After the TMS/pinch experiments were complete, subjects were instructed to move to an adjacent room where a BrainSight program was used on an Apple iMac to validate the TMS stimulation site. Subjects wore a set of glasses, which had mounted sensors on them that could be sensed by a Polaris camera placed in front of them. The Polaris camera is able to use infrared technology to track the three dimensional location of the subject (via the glasses) in space, and the relative locations between the subject’s head and the coil tracker or pointer.

The steps were: landmark calibration, validation, and online marking of the pointer and TMS coil (see Figure 7 for the pointer, glasses, and mounted TMS coil and for the setup with the Polaris camera).

**Figure 7:** This figure shows the setup of the post-experiment marking of the stimulated area DLPFC using Brainsight technology. Subjects wore a set of clear glasses with a mounted subject tracker, and the experimenter used the calibrated pointer and coil to mark the DLPFC, and the POLARIS camera tracks their relative locations in three-dimensional space and registers this with the subject’s three-dimensional brain project.
First, each subject had to go through a calibration process, where the pointer was used to pinpoint the locations of various landmarks on each subject’s head, and these locations were captured by the Polaris camera in relation to the subject (via glasses).

Next, the pointer was placed in front of, behind, to the left of, and then to the right of the subject’s head to ensure that the orientation of the calibration was correct; during this process, a second experimenter monitored the location of the pointer relative to the 3D reconstructed brain on the computer screen. Then, the location of the left DLPFC, marked on the scalp as 5 cm anterior to the M1 hotspot, was captured by placing the tip of the pointer on the marking, which Polaris captured.

Finally, the 40 mm conditioning TMS coil, of which a BrainSight sensor was attached to, was placed on the scalp of the subject in the same orientation and location that it was placed in during the experiment during the dual site recordings. This was captured also by Polaris.

When evaluating the stimulation location of each subject during offline analysis, each location marked (pointer vs. coil) on the scalp were projected perpendicularly on the scalp downwards onto the cortical surface of the brain. This trajectory would yield the center of the stimulation area on the brain, as recorded by the coil (stimulation site) and pointer (scalp-marked location of DLPFC). Next, the smallest distance between the pointer-location on the brain and the coil-location on the brain was obtained on Brainsight, which automatically computes the minimal trajectory distance. This distance was noted, and the average Talairach and MNI co-ordinates of the coil position were also recorded and averaged. The reference MNI co-ordinates of the left DLPFC from the literature was obtained from a resting state fMRI study by Watanabe et al. (2013) to be (-48, 21, 38; MNI co-ordinates). See Figure 8 for a schematic of DLPFC localization on Brainsight.
Figure 8: The figure above shows the 3-dimensional brain project, which is a compilation of the slices from a subject’s anatomical MRI scan. Green crosshairs indicate for every view (coronal, sagittal, transverse) the co-ordinates of the projected marking from the scalp (green TMS coil) down to the brain.
3.4: Analysis: Electrophysiological Data

Motor-evoked potential (MEP) data was collected through Signal program (Cambridge Electronic Design, Signal Version 4.07), and these files were transferred to a safe storage site for offline analysis. The three steps to the electrophysiology data analysis include a pre-processing stage, a processing stage, and a statistical stage.

**Pre-processing stage:**

Signal .cfs files were processed with a script that calculated the peak-to-peak amplitude of each trial frame in mV. These values were tabulated by trial number and muscle channel in an output .txt file, and were saved for further analysis.

**Processing stage:**

All electrophysiological .txt files were imported into a MATLAB program (MATLAB Version R2013a, Mathworks Inc.). If it was noted during the TMS experiment that specific frames should be deleted (due to coil moving off of stimulation site, or if subject had voluntary muscle movement, for instance), an option was made available here to remove those frames.

Next, the averages and standard deviations of each state was calculated (i.e. test pulse only, ISI=2, 3, 10, 15 ms). There were 8 trials for each state. The individual frames of a particular state were then compared to their respective averages, and if a frame was greater than 2 standard deviations above the mean or less than 2 standard deviations below the mean, these frames were removed and excluded from the statistical analysis. After the outlier frames were removed, a new average for each state was calculated.

Next, the averages for each of ISI (i.e. 2, 3, 10, 15 ms) were “normalized” by dividing these average values by the average amplitude of the test MEP alone, in order to determine whether facilitation or inhibition occurred in these paired pulse/dual site conditions compared to when M1 was stimulated alone. The processing step was repeated for all electrophysiological recordings at the subsequent TMS stages.
Statistical Stage:

First, a repeated measures ANOVA was conducted to determine the effects of condition (sequence or random) and also the effect of training (PRE, DURING, POST 0, POST 30) on the neurophysiological measures. Then, a 2x4 repeated measure analysis of variance (ANOVA) was conducted to determine whether a significant interaction existed between “sequence” (i.e. SEQ visit, RANDOM visit) and “training” (i.e. PRE, DURING, POST 0, POST 30). A p-value of 0.05 was considered significant.

Analysis: Behavioural Data

From the designed Labview program, the values collected during trials are the positions of the cursor, sampled at a 50 Hz frequency. The output file detailed these values in an Excel spreadsheet, with 120 rows of position values for every block of training. Since every trial is not of the same duration (i.e., some trials took longer and some took a shorter time), the shorter trials had trailing cells with “0” as the value to signify the trial terminated, and the purpose for this is to ensure that the entire output file had the same number of values in the columns (i.e., the output is a matrix).

These values were imported into MATLAB, where a script was designed to process these files for each block of training to yield the following outcome measures: response initiation time (RT), movement time (MT), binary accuracy, the % of correct trials in a block), and spatial accuracy. The exact details of how each outcome measure was determined will be explained in subsequent sections. Prior to obtaining these values, a pre-processing step was done to remove invalid trials.

Pre-processing:

A trial is considered invalid if force generation occurred prior to the cue onset, or if the position of the cursor was not in the home position prior to the cue onset. This could happen if the subject was still squeezing the transducer after the previous trial had elapsed and a new trial had begun. Another type of trial to exclude was the fluctuating trial, where in a given trial the cursor had moved only slightly out of the HOME position and returned HOME, ending the trial. This would occur if calibration was not accurate, or if the subject was readjusting the position of their fingers.
on the transducer, unaware that the trial was being recorded. As these were not goal-directed trials, they were removed. To determine the criteria to reject these invalid trials, we took the average of all position points sampled in each of these trials. If the trial ended prematurely, most of the data values were 0, and thus the average was also close to 0. We evaluated a set of identified “prematurely ending trials” and determined that a value of 0.005 was a suitable threshold value to use to reject a trial—if the average of all of its data points fell below this threshold. After this pre-processing stage, the outcome measures were determined. Measures were calculated both with targets considered separately and also with targets considered together (See Figure 9 for a flowchart explaining the calculations for a particular outcome measure for one training condition (i.e., SEQ or no SEQ).

**Figure 9:** The above schematic shows the concept of calculating individual vs. group averages for a particular outcome measure for 1 block. The above process was repeated for the remaining 9 blocks as well. Measures were also calculated with consideration of separate targets. All behavioural outcome measures were calculated this way with the exception of binary accuracy, since individual trials would only yield a binary outcome (hit or miss). Therefore, only individual and group averages for binary accuracy were calculated.
**Response Initiation Time:**

Response initiation time (RT) was calculated as the time from cue presentation to the time when the subject had moved the cursor to a position where they achieved 5% of the peak velocity in a trial. The peak velocity was derived by using the derivative function in MATLAB for each subject’s individual trials (i.e., the derivative of position would yield velocity). 5% of this value was taken as the threshold for the RT. See Figure 10 for the illustration of RT calculation for a single trial. The grand average RT (for all 120 trials) and the separate target RT (average of targets 1, 2, 3, and 4 separately) were calculated for every block of training for each subject, and the grand average and separate target RT’s for subjects were averaged, into a “group grand average” of all subjects, to observe the average change in RT across blocks with training.

**Movement Time:**

MT is calculated as the time from force generation onset to force cessation. The time of force cessation is defined as the time when the velocity of the cursor fell below 5% of the peak velocity. These MT values were then averaged per trial in every block, to yield the block averaged MT. The averages per block were also calculated separately by target as well. Next, these blocks of MT values were averaged across subjects to obtain a “group grand average” of MT for all subjects across 10 blocks for the SEQ and RANDOM conditions separately. See Figure 10 for the illustration of MT calculation for a single trial.

**Binary Accuracy/Error:**

Binary accuracy is calculated as the percentage of trials in which the subject successfully reached the target, divided by the total number of trials in that block after error trials were rejected. Binary error is the difference of this value from 1. The possible range of position values for the cursor range from 0 (HOME) to 1.25 (very top edge of the screen). The positions of the targets are 0.25, 0.5, 0.75, and 1 for targets 1, 2, 3, and 4, respectively. The size of the targets and cursors were taken into consideration during this calculation of accuracy, and the condition for a “hit” to occur is that the peak position of the cursor trajectory during a particular trial must satisfy the following criteria: the cursor’s edge must at least be touching the edge of the cued target. If this condition is not met, the trial is considered a “miss”.
Figure 10: The schematic above shows an exemplar of a single trial mapping the position of the cursor over time. The Y-axis value of 0 is the “HOME” position, and force generation linearly moves the cursor upward on the computer screen. In this example, Target 2 was the cued target, of which the target center is situated at the position 0.5. Points A and C are the points at which the cursor’s position had reached 5% of peak velocity in the trial, and Point B was the peak position reached by the cursor. The difference in time between Points C and A yield the MT, and the time from cue presentation to Point A yields the RT. Point B is evaluated based on the margin of error allowed (i.e. not undershooting or overshooting the target), and binary accuracy was calculated.

Spatial Accuracy: Spatial accuracy was calculated by determining the distance between the center of the cursor at its peak position during a trial and the center of the cued target, on a trial by trial basis. The value obtained can be thought of as a measure of accuracy, and an average of these values in a block can be obtained to see whether or not the accuracy improves over time (a value approaching 0 would indicate the subject is able to move the cursor closer to the center of the target on a more consistent basis). The change in this accuracy value over blocks can be used
as a measure of sequence acquisition. All pre-processed trials were included in the calculation of spatial accuracy, including both “hit” and “miss” trials.

Skill Measure (Accuracy/RT & “Knee” Value):

Through observing the initial behavioural results, we used various approaches to characterize a skill measure. First, we wanted to confirm that a speed-accuracy trade-off exists in our data. To confirm, plots of the binary error (1-binary accuracy) values against the average MT were created for each subject per block, to determine if we would reproduce the characteristic trade-off curves.34

After testing several models, we chose to represent the data in two particular models.

The first model incorporated both speed and accuracy into a single variable by taking a ratio of the binary accuracy (expressed as 0-1, with “1” representing perfect accuracy, all “hits” and no errors) divided by the RT. A high accuracy with a short RT would yield a large skill value; a low accuracy with a long RT would yield a small skill value; a high accuracy with a slow RT or a low accuracy with a fast RT would yield intermediate skill values (see table provided).

<table>
<thead>
<tr>
<th>Accuracy/RT Skill Measure</th>
<th>RT Fast (LOW value)</th>
<th>RT slow (HIGH value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy LOW</td>
<td>Intermediate skill value</td>
<td>LOW skill value</td>
</tr>
<tr>
<td>Accuracy HIGH</td>
<td>HIGH skill value</td>
<td>Intermediate skill value</td>
</tr>
</tbody>
</table>

Table 2: The table above shows the consequence of taking the ratio of the binary accuracy divided by the RT. A ratio with a high accuracy and a fast RT will yield a higher skill value, whereas a low accuracy with a slow RT will yield a low skill value.
The second model incorporated MT and accuracy into a single variable. We first plotted the average MT against the binary accuracy rate, separately for SEQ and RANDOM conditions, to validate that there indeed was a speed accuracy trade-off. After confirming the speed-accuracy trade-off, we computed the Euclidean distance, or the shortest distance from the origin (0, 0) on the trade-off graph to each of the points on a trial-by-trial basis for each subject, and termed this the “knee” value (See Figures 18 and 19 for the trade-off graphs and for a sample calculation of the Euclidean distance). Theoretically, subjects who display increased accuracy and faster MTs would be individuals who show sequence acquisition. Therefore, on this trade-off curve, points that are closest to the origin where there is minimal error and the fastest MT would indicate the highest skill. We averaged these initial “knee” values on a trial-by-trial basis for each subject per block, and took the reciprocal of these averages. This final “Knee” reciprocal value would reflect the skill measure, with a higher value indicating higher skill. Next, this new Knee value average was averaged across subjects and we observed how this group-averaged “Knee” value changed across the 10 blocks of training.

**Statistical Analysis:**

The group block averages for each outcome measure (RT, MT, accuracy), either with targets calculated together or separately, all underwent the same type of repeated measures ANOVA analysis using SPSS [IBM SPSS Statistics 21.0] to look at whether main effects of condition and training were significant, and whether there were significant interactions between “sequence” (either SEQ or RANDOM) and “training” (2x8). In this analysis, “training” was the averaged outcome measures from block 1 to 8 (8 variables). We also looked at the effects of “task switching” from the sequence block to the random block, which included the averaged outcome measures from block 8 (sequence) and 9 (random) (2 variables). Furthermore, we looked at whether there were significant interactions between condition and task-switching (2x2). The reason why we tested these groups were because we were interested in whether sequence training outcome measures changed in a similar pattern compared to random training (2x8 test). We were also interested to see if task switching in the sequence condition, from block 8 to 9, would change the outcome measures compared to in the random condition, where blocks 8 and 9 were both random. Evaluating whether interactions exist from block 8 to 9 would allow us to examine at whether the change from a sequence block to a non-sequence block in the SEQ visit has a
significant effect on the outcome measures compared to a change from a random block to another random block in the random condition, which serves as a control.

When considering the significance of a specific ANOVA test, first Mauchly’s test of sphericity was conducted to test whether the variances of the differences between all combinations of related groups (levels) are equal (Laerd Statistics, SPSS, IBM). If a data set passed the sphericity test (i.e. p-value is not significant), the p value that was computed assumes sphericity of the data. If the data set failed the sphericity test (i.e. p-value was significant, p<0.05), the Greenhouse-Geisser correction was applied, and the corrected value of P<0.05 was taken as the value of significance in the repeated measures ANOVA.

**Multivariate analysis (Correlations)**

Pearson correlation analysis was used to determine whether a correlation existed between age and the changes in electrophysiological measures with training, and between age and the changes in behavioural measures with training. P values < 0.05 were considered significant correlations (See Table 3 below).

Pearson correlation analysis was used to determine whether correlations existed between the changes in electrophysiological measures and the changes in behavioural measures with motor pinch training. P values < 0.05 were considered significant correlations (See Table 3 below).
Correlations Tested (Pearson Correlation)

<table>
<thead>
<tr>
<th>Measure 1</th>
<th>Measure 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Does age correlate with changes in behavioural measures with motor training?</strong></td>
<td>Age</td>
</tr>
<tr>
<td><strong>Does age correlate with sequence-specific sequence acquisition in the behavioural measures?</strong></td>
<td>Age</td>
</tr>
<tr>
<td><strong>Does age correlate with changes in TMS measures with training?</strong></td>
<td>Age</td>
</tr>
<tr>
<td><strong>Do changes in behavioural measures with training correlate with changes in electrophysiological measures with training?</strong></td>
<td>RT, MT, spatial accuracy, binary accuracy, and Accuracy/RT skill measure</td>
</tr>
<tr>
<td><strong>Do changes in behavioural measures that are sequence-specific correlate with changes in electrophysiological measures with training?</strong></td>
<td>RT, MT, spatial accuracy, binary accuracy, and Accuracy/RT skill measure</td>
</tr>
</tbody>
</table>

Table 3: The table above shows all of the Pearson Correlation tests that were performed. The first column indicates the associations that were tested; the second column indicates the first measure used in the correlation; the third column indicates the second measure used. Abbreviations: RT=response initiation time, MT=movement time, SICI=short intracortical inhibition, ICF=intracortical facilitation, DLPFC-M1=dorsolateral prefrontal cortex-to-primary motor cortex, MEP=motor evoked potentials.
CHAPTER 4: RESULTS

The age of all 14 subjects was 40±18.2 years (standard deviation) (range=23-68 years, 6 females and 8 males). Subjects all reported themselves to be right-handed and this was confirmed by Edinburgh’s Handedness Inventory.

Behavioural Data

Sequence recognition:

For the SEQ visits, in which subjects unknowingly trained in the pinch task with a repeating 12-item sequence, 9 of the subjects did not report noticing a repeating pattern throughout the entire training. 5 subjects noticed a repeating pattern, but stated that they could not recall or reproduce the 12-item sequence.

Outcome Measures:

RT decreased across the 10 blocks for both SEQ and RANDOM conditions, and this pattern persisted even when the RT was analyzed separately by targets (See Figure 11). From blocks 8 to 9, there was an increase in RT in the SEQ condition, but not the RANDOM condition, in the all-target analysis. In the separate-target analysis, this pattern existed for Targets 3 and 4 (the furthest 2 targets), but not for Targets 1 and 2. Repeated-measures ANOVA analysis on group RT, for condition (SEQ vs. RANDOM) x blocks (blocks 1-8) showed a significant effect of blocks 1-8 on the resulting RT (F=8.714, p<0.001, Greenhouse-Geisser), but not condition (F=0.584, p=0.461, Greenhouse-Geisser). There was no significant interaction of condition x blocks 1-8 on the resulting RT (F=1.021, p=0.390, Greenhouse-Geisser). RM-ANOVA for condition and random transfer (blocks 8, 9, 10) showed no significant effects of condition or random transfer on RT, but there was a significant interaction between conditioning and random transfer, together on RT (F=4.186, p=0.027, Sphericity Assumed).

When the RT values (all targets) for each block were normalized against Block 1, the individual target analysis showed similar patterns, except that the normalized RT values for the RANDOM condition eventually returned to baseline values with training, and the SEQ condition normalized RT’s remained below baseline. For blocks 8-10, there was a trend for significant effect of
training on the normalized RT for the all-target analysis (F=2.810, p=0.082, Sphericity Assumed). There was a significant effect of training on the overall normalized RT over blocks 2-8 (F=3.554, p=0.004, Sphericity Assumed).

Figure 11: The above schematic shows the averaged raw RT data across the 10 blocks of training (expressed in seconds), for both the SEQ (green plot) and the RANDOM (red plot)
visits. Figure A shows the RT graph with all the targets combined, and Figures B-E show the individual targets. Block 9 did not consist of a sequence for the SEQ visit, but instead had a completely randomized target order. Error bars are standard error of the mean.

**: p<0.01, ANOVA effect of training

^: p<0.05, ANOVA effect of training x condition.

For the target-separate analysis, training from blocks 1-8, once again, showed a significant effect on the normalized RT. For the analysis for blocks 8-10, the increase from block 8 to 9 was, again, most apparent for Target 3 (F=4.212, p=0.007, Greenhouse-Geisser) and Target 4 (F=3.67, p=0.039, Sphericity Assumed) (see Figure 12).

![Graphs A-D](image)

Figure 12: Graphs A-D above show the average normalized RTs of the subjects (normalized against block 1) for all 4 targets. Error bars are standard error of the mean.
Movement Time:

For the all-targets analysis, training (from block 1 to 8) had a significant effect on the MT (F=5.517, p=0.008, Greenhouse-Geisser), but there was no significant interaction between condition, training, and MT.

For the target-separate analysis, the graphs (Figure 13) showed a general decrease in MT with training for both conditions, with the RANDOM condition showing slightly more decreases, although this was not statistically significant. When testing blocks 1-8, there is only a significant effect of blocks on the MT. When testing blocks 8-10, there is no significant effect of condition and random transfer on the resulting MT. See Figure 13 for the separate target graphs of MT.
Figure 13: The above graphs show the all-targets averaged MT for the training task across 10 blocks (Figure 13A), as well as the separate-target graphs for the four targets with training (Figures B-E). Error bars are standard error of the mean.

**: p<0.01, ANOVA effect of training
Accuracy:

The averages for binary accuracy for both SEQ and RANDOM conditions remain fairly consistent with training and repeated measures ANOVA yielded no significant influence of either condition, training, or condition x training on the resulting accuracy. See Figure 14 for the graph detailing binary accuracy in the experiments.

Spatial Error:

The target-separate graphs for spatial error are displayed in Figure 15. Overall, the spatial error remained relatively consistent across training blocks for both SEQ and RANDOM conditions. There is a trend for the spatial error in SEQ condition to be lower with training compared to RANDOM, especially for Target 4, though this did not reach significance. Figure 16 shows a sample graph of Target 4, with the values represented on the y-axis being the normalized spatial error against the first block. This graph shows that for the SEQ, the overall spatial errors, and their variation (represented by the standard deviation), remain near baseline values across the training blocks, whereas the RANDOM condition showed an increased degree of error and variability over training.

Figure 14: The figure above shows the average binary accuracy for all subjects across the 10 blocks of training. Error bars represent standard error of the mean. Green plots represent averages from the SEQ condition, and red plots represent averages from the RANDOM condition.
Figure 15: The figures 15A-D above shows the averaged spatial error across 10 blocks for all subjects, separated by target. Error bars represent standard error of the mean. Green plots represent averages from the SEQ condition, and red plots represent averages from the RANDOM condition.

Figure 16: The figure above shows a sample of one target’s spatial error (target 4), normalized against block 1. Error bars represent standard error of the mean. Green plots represent averages from the SEQ condition, and red plots represent averages from the RANDOM condition.
*Skill Measure:*

We obtained the averaged skill measure value of each subject and obtained the group skill measure for both SEQ and RANDOM conditions (through taking a ratio of the binary accuracy and the RT). There is an overall increase of skill measure in both conditions between blocks 1 to 3, indicating skill improvement (i.e. more accuracy, faster RT) (F=5.784, p=0.008, Sphericity Assumed). Training (Blocks 1-8) improved skill measure for both groups (F=3.092, p=0.05, Greenhouse-Geisser). For the SEQ visit, when tested with a random block at block 9, the skill measure value returned back to baseline levels, and at block 10 the skill measure increased again. Blocks 8-10 for the RANDOM condition remained relatively stable. A repeated-measures ANOVA conducted for blocks 8-10 showed a significant interaction between condition and training on skill measure (F=5.11, p=0.013). See Figure 17 below for the plot of the Accuracy/RT skill measure with training.

*Figure 17:* The above graphs shows the group averaged skill measure values at each block of the motor pinch training for the SEQ and RANDOM conditions. The green plot represents the SEQ condition, and the red plot represents the RANDOM condition. Error bars represent standard error of the mean. \(^\wedge: p<0.05, \text{ANOVA effect of condition x training}\)
MT and error (“Speed-accuracy”) trade-off:

A speed-accuracy trade-off was plotted for the RANDOM and SEQ visits separately, and a characteristic trade-off curve was observed (Figures 18 & 19). Theoretically, subjects who display increased accuracy and faster MTs would be individuals who show sequence acquisition. Therefore, on this trade-off curve, a plot closest to the origin (where there is minimal error and the fastest MT) would indicate the highest skill. Through calculating the Euclidean distance for each data point in each of Figure 18 and 19, we computed the shortest distance from the origin (0, 0) to the points (MT, binary error) on a trial-by-trial basis for each subject, and termed this the “knee” value, based on the trajectories drawn to yield Euclidean distance (see Figure 18). We averaged these “knee” values across subjects and observed how they changed across the 10 blocks of training. According to Figure 20, subjects began at a similar level of “knee skill measure” at block 1 of training, but improvements were seen in between blocks 1-3 for the RANDOM visit, and not the SEQ condition; the knee value in block 4 caught up for the SEQ condition. A RM-ANOVA tested the initial effects of training (blocks 1-4) and condition (SEQ vs. RANDOM) and found a significant effect of training x condition was observed (F=4.375, p=0.010, Sphericity Assumed). Through blocks 4-8, both SEQ and RANDOM improved gradually in parallel. When tested at block 9, the average Knee value for the SEQ visit decreased, while the RANDOM values did not. The RM-ANOVA was conducted to determine the effects of random transfer (blocks 8-10) and condition (SEQ vs. RANDOM) on the resulting knee skill measure value. There were no significant effects of either random transfer or condition on the resulting knee value. However, there was a significant interaction of random transfer x condition (F=3.478, p=0.047, Sphericity Assumed).
Figure 18: The graph to the left shows a plot of error rate vs. MT (seconds) for subjects in the RANDOM visit. Each plot colour represents a single subject at a particular block (for a total of 10 blocks, 10 plots for each colour). Error rate was measured as the percentage of incorrect trials divided by the total number of valid trials in a particular block.

Figure 19: The graph to the left shows a plot of error rate vs. MT (seconds) for subjects in the SEQ visit. Each plot colour represents a single subject at a particular block (for a total of 10 blocks, 10 plots for each colour). Error rate was measured as the percentage of incorrect trials divided by the total number of valid trials in a particular block.
Figure 20: The graph above shows the calculated “Knee” skill measure value across the 10 training blocks. Each data value was obtained by taking the reciprocal of the average Euclidean distance between the MT and error value for subjects, and taking the group average of these values. A higher Knee value represents higher school here. Green plots indicate average values for the SEQ visit; red plots indicate average values for the RANDOM visit. Error bars represent standard error of the mean.

^: p<0.05, ANOVA effect of condition x training;  **: p<0.05, ANOVA effect of training

Brainsight/Neuronavigation:

The MNI co-ordinates averaged across subjects for the site of left DLPFC stimulation were found to be: x = -25.3 ± 7.0 [range = (-35.0, -17.4)], y = 29.5 ± 9.7 [range= (14.0, 47.9)], z = 39.1 ± 6.8 [range= (29.4, 50.7)].

Electrophysiology Results

Baseline Values for TMS Recordings:

Figures 21 and 22 show the averaged baseline recordings for the different TMS measures, with the purpose of comparing the baseline values for the SEQ and random visits. The baseline averages across subjects for each measure were not significantly different for the sequence and random conditions (paired t-test).
Figure 21: The graph on the left shows the averaged baseline motor-evoked potential for all subjects at the SEQ and RANDOM visits, prior to engaging in motor pinch training. The graph on the right shows the baseline averaged MEP for all subjects at the SEQ and RANDOM visits for the paired pulse recordings, at the various ISI’s. The red bar labelled “R” represents the average for the RANDOM visit; “S” represents the average for the SEQ visit. Error bars represent standard deviation. rFDI = right first dorsal interosseus muscle.

Figure 22: The graph on the left shows the baseline averaged MEP for all subjects at the SEQ and RANDOM visits for the dual-site recordings (DLPFC-M1) at various ISI’s, with the conditioning coil intensity at 90% of RMT. The graph on the right shows similar data, but with the CS coil at 110% of RMT. The red bar labelled “R” represents the average for the RANDOM visit; “S” represents the average for the SEQ visit. Error bars represent standard deviation.
**M1 Excitability**: The overall excitability of the primary motor cortex remained the same for the RANDOM visit until 30 minutes after training, where there was facilitation. For the SEQ condition, there was inhibition after 2 blocks of training and also immediately after training, but after 30 minutes of training, there was facilitation as well. We then conducted a repeated-measures ANOVA, which showed no significant effects of condition on the normalized FDI MEP or significant blocks x condition interactions on the normalized FDI MEP amplitude. There was, however, a significant effect of training alone on the FDI MEP (F=3.468, p=0.043, Sphericity assumed). The Figure below illustrates the changes in M1 excitability in both conditions with training.

![Graph showing changes in M1 excitability](image)

**Figure 23**: The graph above shows the changes in normalized right FDI MEP at the different stages of motor training. The green plots indicate normalized MEP in the SEQ visit, and red plots indicate normalized MEP in the RANDOM visit. Error bars indicate standard error of the mean. **: p<0.05, ANOVA effect of training
**Paired-Pulse TMS:** Repeated measures ANOVA for SICI at 2 ms and 3 ms, and ICF at 10 ms and 15 ms showed no significant effect of blocks or condition on the normalized FDI MEP. Furthermore, there were no significant blocks x condition interactions. See Figure 24 below for the graphs of the SICI and ICF changes with motor training.

![Graphs A-B](image1)

**Figure 24:** Graphs A-B above show the changes in SICI at ISI= 2 ms and 3 ms with motor training, and graphs C-D show the changes in ICF at ISI= 10 ms and 15 ms with motor training. The green plots represent group averages at different training stages for the SEQ visit; red plots represent group averages for the RANDOM visit. Error bars represent standard error of the mean.

**Dual-site TMS:** Paired dual-site TMS for DLPFC-M1 connection at 90% of resting motor threshold for DLPFC yielded no significant effects of training, condition, or training x condition interaction on the normalized FDI MEP at all the ISI’s tested (4 ms, 6 ms, 8 ms, 10 ms). See Figure 25 below.
Figure 25: The graphs A-D above show the dual site TMS electrophysiology results. The conditioning coil over the left DLPFC was set to a stimulation output of 90% of resting motor threshold, and the ISI varied between 4, 6, 8, and 10 ms. Green plots detail the normalized RFDI values during the SEQ visit; red plots detail the RANDOM visit. “During” indicates a time point after 2 blocks of the motor pinch training. Error bars represent the standard error of the mean.

Repeated measures ANOVA on the DLPFC-M1 connectivity tested at 110% of resting motor threshold showed no significant effects of condition or time on the resulting DLPFC-M1 MEP. However, there was a significant condition x time interaction at the ISI=8 ms (F=3.402, p=0.027, Sphericity assumed), indicating that at this particular ISI, that time alone cannot explain the changes in DLPFC-M1 connectivity (in essence, that the “condition” had an influence). The p-values were not significant at the other time points (i.e. ISI= 4, 6, and 10 ms, refer to Figure 26).
Figure 26: The graphs A-D above show the dual site TMS electrophysiology results. The conditioning coil over the left DLPFC was set to a stimulation output of 110% of resting motor threshold, and the ISI varied between 4, 6, 8, and 10 ms. Green plots detail the normalized RFDI values during the SEQ visit; red plots detail the RANDOM visit. “During” indicates a time point after 2 blocks of the motor pinch training. Error bars represent the standard error of the mean.\(^*\): \(p<0.05\), ANOVA effect of condition x training.
Correlation Results

Age correlations:

There was a significant positive correlation between age and the changes in RT as tested by taking the difference between blocks 9 and 8, in the SEQ visit (Pearson Correlation=0.744, p=0.002, N=14). That is, with increased age, there was greater increase in RT with the random block at block 9. There was no significant correlation in the RANDOM condition (Pearson Correlation=0.48, p=0.082, N=14). The data is shown in Figure 27.

Figure 27: The figure above displays the relationship between age and changes in RT (in seconds, taken as the difference between block 9 and block 8). Significance is indicated with asterisks (**, p<0.01). Green plot = SEQ condition, Red plot = RANDOM condition.

When examining the relationship between age and the changes in SICI (2ms ISI) after motor training (difference between values at 0 min after training and at baseline), there was no
significant correlation (SEQ condition, [Pearson coefficient= -0.264, p=0.361]; RANDOM condition, [Pearson coefficient= -0.098, p=0.739]). That is, there was no relation between age and the changes in short interval intracortical inhibition (SICI) following training (see Figure 28). Similar results were found when testing the correlation between age and changes in SICI at 3 ms (SEQ: Pearson Correlation=0.648, p=0.134; RANDOM: Pearson Correlation=0.401, p=0.155), ICF at 10 ms (SEQ: Pearson Correlation= -0.086, p=0.770; RANDOM: Pearson Correlation=0.332, p=0.247), and ICF at 15 ms (SEQ: Pearson Correlation=0.002, p=0.994; RANDOM: Pearson Correlation=0.172, p=0.556). Figure 28 below shows the relationship between age and SICI at 2 ms; since there was also no significant correlation between age and the remaining paired pulse measures, these graphs are not shown.

Figure 28: The figure plots age versus the changes in SICI at 2ms with motor training (taken as the difference between the baseline SICI and the SICI immediately after motor pinch training). Each point represents one subject and their respective average difference: green plots represent subjects during their SEQ visit and red plots show subjects during their RANDOM visit.
The correlation between age and changes in DLPFC-conditioned MEP were studied, and a statistically significant, moderately strong, negative correlation was found at a conditioning stimulus intensity of 90% RMT, ISI=4ms, for the SEQ visit (Pearson correlation= -0.678, p=0.008, linear regression $R^2=0.459$), and not for the RANDOM visit (Pearson correlation= -0.459, p=0.099, linear regression $R^2=0.211$). See Figure 29 below shows this relation.

Figure 29: The figure above shows the relationship between age and the changes in DLPFC-Conditioned M1 MEP from baseline, immediately after training. The settings for the conditioning stimulus was 90% of resting motor threshold, and the ISI between CS and TS is 4 ms. Red plots indicate the values for subjects during the RANDOM condition, and green plots indicate values during the sequence condition. Significance is indicated with asterisks for the green SEQ dataset (**, $p<0.01$).
**TMS/Behavioural Correlations:**

We explored whether or not there was a relationship between the changes in RT with training (measured as difference between block 8-1) and the changes in the DLPFC-conditioned M1 MEP with training (measured as difference between POST 0 and PRE). There was a significant positive correlation in the RANDOM condition (Pearson Correlation=0.564, p=0.036, linear regression $R^2=0.318$) but not in the SEQ condition (Pearson Correlation=0.002, p=0.995, linear regression $R^2=0.119$). Figure 30 below shows the correlation with the linear regression line of best fit.

![Figure 30](image)

*Figure 30: the figure above shows the relationship between the changes in RT (block 8 – 1) and the changes in DLPFC-Conditioned M1 MEP (taken as difference between FDI MEP recordings at baseline and immediately after training). A negative value on the x-axis indicates an improvement in RT (block 8’s RT is lower than block 1). Negative values on the y-axis indicate inhibition compared to baseline, and positive values indicate facilitation. Red plots indicate*
values from subjects in the RANDOM condition, and green plots indicate values from the SEQ condition. Significance is indicated with asterisks for the red SEQ dataset (*, p<0.05).

We then examined whether sensitivity to the sequence (tested as the difference between block 9 and 8) correlated with the changes in DLPFC-M1 connectivity. We found a significant negative correlation for DLPFC-M1 connectivity at ISI of 4 ms and at conditioning intensity of 90% RMT for the sequence condition (Pearson Correlation=-0.600, p=0.023, linear regression R²=0.36), but not for the RANDOM condition (Pearson Correlation=-0.28, p=0.351, linear regression R²=0.073). Figure 31 below shows relationship between change in RT and change in DLPFC-M1 connectivity and the regression lines for RANDOM and SEQ conditions.

![Relationship between Changes in DLPFC-M1 MEP (ISI=4 ms, 90% RMT) and RT (Block 9-8)](image)

Figure 31: The graph plot above shows the relationship between the changes in RT (tested as block 9-8) and the changes in DLPFC-M1 connectivity at ISI of 4ms conditioning intensity of 90% RMT. Red plots indicate average differences in the RANDOM visit and green plots indicate values from the SEQ visit. Significance is indicated with asterisks for the green SEQ dataset (*, p<0.05).
There were no significant correlations between MT changes and changes seen in the electrophysiological measures after training.

We next looked at the changes in spatial accuracy with training to determine whether changes in this parameter correlated with changes in neurophysiological measures. Significance was found when correlating spatial accuracy (block 8 – 1) with DLPFC-M1 connectivity at conditioning intensity of 110% RMT (POST 0 – PRE) for the following ISIs: 8 ms for RANDOM (Pearson correlation= -0.650, p=0.012, linear regression $R^2=0.423$), and 10 ms (RANDOM Pearson correlation = -0.654, p=0.011, linear regression $R^2=0.427$), (SEQ Pearson correlation= -0.606, p=0.022, linear regression $R^2=0.367$). Figure 32 shows the changes in spatial accuracy against changes in DLPFC-M1 connectivity at 8 and 10 ms ISI’s for both SEQ and RANDOM conditions.

**Figure 32:** The graphs to the left show the changes in spatial accuracy (difference between block 8 and 1) and the changes in DLPFC-M1 connectivity after training (difference between POST 0 and PRE). A negative x-axis value indicates improvement in spatial accuracy after 8 blocks. A positive number on the y-axis indicates facilitation after training, and a negative value indicates inhibition. Red plots are the values for subjects in the RANDOM visit; green plots are values for subjects in the SEQ visit. Top graph is the plot at ISI= 8 ms, and bottom graph is a similar plot with ISI=10ms.Significance is indicated with asterisks for the red and green SEQ datasets (*, $p<0.05$).
Next, we examined the changes in spatial accuracy in relation to the electrophysiology measures. Through correlating spatial accuracy changes (block 9-8) and changes in DLPFC-M1 connectivity, we found a significant correlation only for the SEQ condition at ISI of 8ms, conditioning intensity of 90% RMT (Pearson correlation= -0.568, p=0.034, linear regression $R^2=0.323$). The correlation was not significant for the RANDOM condition (Pearson correlation = -0.172, p=0.557, linear regression $R^2=0.03$). Figure 33 below shows the correlation for both conditions and the linear regression lines.

![Graph showing correlation between DLPFC-M1 MEP and spatial accuracy](image)

**Figure 33:** The graph above shows the changes in spatial accuracy (difference between block 9 and 8) and the changes in DLPFC-M1 MEP after training (difference between POST 0 and PRE) at ISI=8 ms and conditioning stimulus intensity at 110% RMT. A negative x-axis value indicates improvement in spatial accuracy after 8 blocks. A positive number on the y-axis indicates facilitation after training, and a negative value indicates inhibition. Red plots are the values for subjects in the RANDOM visit; green plots are values for subjects in the SEQ visit. Significance is indicated with asterisks for the green SEQ dataset (*, p<0.05).

Finally, we studied the correlations between changes in skill measure and changes in electrophysiological measures. When comparing the overall difference of the accuracy/RT skill
measure from training (block 8-1) with the change in DLPFC-M1 connectivity (POST 0 – PRE), at different conditioning stimulus intensities (90% RMT and 110% RMT) and the different ISIs (4 ms, 6 ms, 8 ms, 10 ms), there were no correlations. When testing the sensitivity of subjects to the sequence through subtracting the skill measure for block 8 from block 9, there was a significant negative correlation between this difference and the changes in DLPFC-M1 connectivity (POST 0 – PRE) at 110% RMT and at ISI of 8 ms for the SEQ visit (Pearson correlation=-0.56, p=0.037, linear regression $R^2=0.314$) but not the RANDOM visit (Pearson correlation=-0.262, p=0.366, linear regression $R^2=0.055$), as detailed in Figure 34 below.

**Figure 34: The graph above shows the changes in skill measure (block 9-8) and the changes in DLPFC-M1 connectivity after training (POST 0 - PRE) at ISI=8 ms and CS intensity at 110% RMT. A negative x-axis value indicates worsened performance at block 9 compared with 8. A positive number on the y-axis indicates facilitation after training, and a negative value indicates inhibition. Red plots are the values for subjects in the RANDOM visit; green plots are values for subjects in the SEQ visit. Significance is indicated with asterisks for the green SEQ dataset (*, $p<0.05$).**
CHAPTER 5: DISCUSSION

The findings of this study suggest that (1) age is related to changes in functional connectivity of the DLPFC to M1, through decreasing motor training-associated changes in M1 excitability; and (2) sequence acquisition is associated with facilitation in the DLPFC-M1 connection, as shown by correlation with changes in RT, spatial accuracy and the accuracy/RT skill measure.

The behavioural results demonstrate the various sequence acquisition outcome measures obtained through the motor pinch task, and also demonstrate that sequence-specific behavioural changes did occur through comparing the behavioural measures of the SEQ and RANDOM visits (as tested at block 9). Through observing the RT values and also through analysing the resulting skill measure (characterized as the “Knee value” and the ratio of accuracy/RT), we verified that the subjects did acquire the sequence, despite most subjects reported that they were not aware of the sequence at the end of their SEQ visit. Therefore, their knowledge of the sequence was still implicit after the training task. It is important to note that our study design only assesses a short timeframe of motor skill acquisition, and that a motor skill would require a longer time to become stabilized in the brain as a motor memory. Thus, we have not established that the subjects have learned the sequence, but rather that brain processes seem to be causing different behavioural outcomes in sequence training versus random practice.

(1) We addressed our first hypothesis that the sequence-acquisition behavioural measures were correlated with changes in DLPFC-M1 TMS measures. There were TMS-behavioural correlations in some behavioural measures, but not others. We compared changes in behavioural measures with changes in electrophysiological measures and found that improvements in spatial accuracy with training were associated with facilitation in DLPFC-M1 connectivity, and that worsened performance at block 9 compared with 8 for spatial accuracy in the sequence condition was associated with facilitation in DLPFC-M1 connectivity. If we take the worsened performance at block 9 (in this case, spatial accuracy) to mean a sensitivity to the sequence change that indicates sequence acquisition, then we can use the degree of worsened performance as a sequence-acquisition measure (i.e., worsened performance at block 9 means that they learned the sequence; no change or improvement at block 9 means they did not learn it). Finally, we also found that acquiring the sequence, as measured in the skill measure (accuracy/RT)
change from block 9 and 8, is associated with facilitation again in DLPFC-M1 connectivity. Of course, correlation does not mean causation between the two variables, but the fact that our results show similar correlations from different behavioural measures strengthens the idea that there is a relationship between the behavioural performances in sequence acquisition and the subsequent changes in DLPFC M1 connectivity as tested by TMS.

Figure 35: Postulated cortico-motor and subcortico-motor pathways that are involved in DLPFC-M1 connectivity during sequence acquisition. Red boxes delineate the hypothesized substrates involved, and arrowheads indicate direction of signal transmission. Coloured arrows indicate the type of neurotransmitter involved in each projection (as indicated in the legend). Original image obtained from https://apackofneurons.wordpress.com/tag/hyperdirect-pathway/
What is particularly fascinating is why this effect is strongest at the particular stimulation intensity of 110% of RMT, and at an interstimulus interval of 8 ms, whereas at 6 ms and 10 ms the trend remains, but is not statistically significant. One possible explanation is that 8 ms is the optimal time for a signal to travel between DLPFC and M1, with the input signal travelling through either cortical areas such as the dorsal premotor cortex or subcortical areas such as the caudate nucleus (See Figure 35). These speculations are based on studies that showed increases in DLPFC-caudate white matter tract integrity after implicit sequence acquisition and increased DLPFC-premotor cortex connectivity after manipulation of spatial sequences. It would be worthwhile to conduct future studies to test these theories—perhaps a combined dual-site TMS study that also examines the premotor cortex to motor cortex connection (not clear what is the relevance of motor conduction time).

**Effects of Training: changes in intracortical inhibition and facilitation**

There was no correlation between the changes in RT with training (block 8-1) and changes in the intracortical facilitation at 10 ms (post 0 – baseline) in the RANDOM and SEQ conditions. Our results agreed with the previous studies that described no change in ICF after motor skill training, while other studies have also reported increased ICF with motor training.

We expected to see decreased SICI with motor training, but we did not obtain these results. There was no significant effect of training or condition on the SICI from repeated measures ANOVA for both SEQ and RANDOM conditions, and with the paired t-test, there was no difference in SICI after training compared to baseline for both the SEQ and RANDOM conditions. This was unexpected, as it would have been expected that motor training, and specifically sequence acquisition, would decrease the intracortical inhibition in the motor system. GABA is synthesized and stored in cortical neurons and is predicted to be an important neurotransmitter in motor sequence acquisition, and SICI is thought to be a measure of the level of GABA-mediated inhibition in the motor-cortical system. A study by Floyer-Lea et al. used a MRS (in-vivo magnetic resonance spectroscopy) and a motor sequence learning task to show that there was a decrease in mean GABA concentration with sequence-specific training, and not in non-specific motor movements. These changes suggest that very rapid, regionally-
specific presynaptic modulation of GABAergic input to M1 facilitates motor learning. Also, Perez et al (2007b)\textsuperscript{195} use SRTT training to show that SICI is reduced in both the trained and untrained M1. A possible explanation why we did not see decrease in SICI in our experiment is the nature of the task itself, which is predominantly dependent on force production, and less on the cognitive recognition of a pattern. A larger proportion of subjects in Perez’s study noticed a repeating pattern, despite none of the subjects being able to regenerate it. Since the number of items in Perez’s study is the same as in our study, adding a force component to the experiment would make the pattern even more difficult to detect, since subjects would be focused on generating the appropriate pinch force to reach the targets. Kantak et al (2012)\textsuperscript{196} provided evidence suggesting that implicit and explicit sequence learning compete with each other in the human motor memory systems through applying anodal transcranial direct current stimulation to M1 (the substrate implicated in implicit motor learning) and the dorsal premotor cortex (a substrate implicated in explicit motor learning) and found that anodal tDCS at M1 during practice significantly improved SRTT practice performance and supported consolidation of the implicitly learned memory. Therefore, M1 is involved in motor memory consolidation, but it is not known whether this consolidation process occurs through cognitive or motor domains. A study detailing the differences in neurophysiology measures in SRTT and SVIPT would be helpful to tease apart the processes that M1 is critical for; perhaps M1 is more involved with detection and processing of sequences than with force gradient generation, of which our pinch task incorporated both domains to increase task difficulty.

The changes SICI, measured as the difference between SICI at baseline to SICI immediately after training did not correlate with age. Previous studies have detailed the importance of a balance between excitation and inhibition in neurons to maintain effective neuronal processing\textsuperscript{197,198}, and changes in GABA-mediated neuronal transmission are important in homeostatic regulation of pyramidal cell phasic inhibition\textsuperscript{199}. Taken together, our negative results seem to suggest that intracortical inhibition is not associated with the improvements in behavioural measures during the sequence acquisition task. There have been studies that support the role of SICI in use-dependent plasticity\textsuperscript{56,60,200–202}, but there are also studies that dispute its involvement or suggest strengthened SICI\textsuperscript{203,204}. Rather, the discrepancies in SICI may be due to the individual experimental settings, i.e. whether SICI was tested with a constant test TMS intensity or whether it was adjusted at each measurement. Studies have also argued that SICI
measures are sensitive to the test TMS intensity\textsuperscript{205}, and are unrelated to cortical excitability state or MEP size\textsuperscript{206}.

In our study, we adjusted our test stimulus such that it matched an average of 1 mV in FDI MEP before each of our recordings. Our results are in agreement with the previous studies in showing no differences in the left hemisphere SICI with motor training, despite M1 experiencing a change in excitability after motor pinch training; therefore, our results are in accord with the notion that use-dependent plasticity in M1 is not attributed to changes or influences in the inhibitory GABAergic neurons.

**Effects of Age**

*Age correlates with neurophysiological measures*

(2) We addressed our second hypothesis that age would correlate with the TMS measures we measured through sequence training. We found no changes in the paired-pulse intracortical measures or the dual site measures. The only age association we found was with the behavioural measure response time. Our electrophysiology paired pulse data showed that overall, when all subjects were pooled together regardless of age, SICI remained unchanged after training at both 2 ms and 3 ms, which is in keeping with previous studies that suggests SICI is not responsible for the reduced use-dependent corticomotor plasticity in older adults\textsuperscript{207}. Also, when correlating each the subjects' age with their changes in SICI after motor training, we observed no correlation between age and the changes in SICI after the motor pinch training task, suggesting that age did not have any apparent relations with the changes in GABAergic intracortical inhibition in M1. This result agrees with Rogasch’s study\textsuperscript{242}; another study by Cirillo et al\textsuperscript{243} also shows that older adults experience no change in SICI in either training hand despite these studies reporting changes in M1 excitability of the right hemisphere after motor training; they argue that increased use-dependent corticomotor plasticity in the right hemisphere was not due to differences in GABAergic intracortical inhibition in M1\textsuperscript{204}. Several studies show no change in SICI with age\textsuperscript{207–209}.

Furthermore, we found an association between age and changes in RT, where older adults experienced larger increases in RT than younger adults when tested at block 9. This is consistent
with task-switching literature, where older adults had a slower response time in the training task than younger adults, and also had larger switching costs (defined as the difference between trials where subjects did not have any switching (no-switch trials) and trials where subject engaged in switching from task A to task B (switch trials))\(^{210}\). Furthermore, one plausible mechanism behind the age-related differences in learning and task switching is that older adults are likely to be more highly susceptible to distraction and that this “distractibility” can serve to either become a cost or benefit, depending on the task at hand\(^ {211}\). If older adults have more difficulty inhibiting irrelevant information and inappropriate responses compared to younger adults, for the purposes of our study, this susceptibility of older adults could be an appropriate explanation as to why their mean RTs and skill measures are significantly slower and worse compared to younger adults. Our study results also associate behavioural improvements in the pinch training task with facilitation in DLPFC-conditioned M1 MEP, which adds to the TMS literature since it has not been previously shown that the “degree of distractibility” or the task switching from sequence to RANDOM is associated with DLPFC-M1 functional connectivity.

(3) **Finally, we addressed our third hypothesis that DLPFC-M1 connectivity is different in sequence acquisition versus random practice.** We were also able to show that sequence-specific acquisition changes DLPFC-conditioned M1 excitability in a different way compared to non-sequence training, specifically at the conditioning stimulus intensity of 110% of RMT. Furthermore, that there is an association between age and the changes in functional connectivity between DLPFC and M1 with motor sequence acquisition. Confirming that implicit sequence acquisition occurred through the behavioural results then allowed us to compare the electrophysiological data in the SEQ and RANDOM visits. This suggests that age increases the sensitivity of individuals to a sequence, despite not explicitly being aware that a sequence was being learnt. Age was also associated with a decrease in DLPFC-M1 excitability changes with motor training, especially when subjects were engaged in the sequence condition. Past studies have looked at changes in prefrontal integrity and function with age: specifically the study by Mizoguchi et al\(^ {244}\) aimed to clarify changes in prefrontal cortex dopaminergic activity in age-related working memory impairment. This study found that aged rats showed reduced dopaminergic transmission in the pre-limbic cortical region of the prefrontal cortex, and that stimulation with a D1 receptor agonist (SKF 81297) improved the age-related working memory, providing direct evidence showing that cognitive deficits develop due to dopaminergic
dysfunction in the normal aging process. This is particularly relevant to our study because we show an age-related change in prefrontal-related corticomotor excitability with motor sequence acquisition, which supports the notion that projections and connections from these prefrontal areas to other areas in the brain (in our case, to the motor areas) change with age and could be the reason why we observed the age-associated changes in the behavioural outcome measures.

DLPFC: The Implicit vs. Explicit Learning Debate

Uncovering the role of DLPFC in goal-directed, sequence-specific learning is important because it is a critical substrate in motor learning; yet, we only know that its role in motor learning is complex, and that it is involved to some degree. Discovering that age changes these connections between DLPFC-M1, and that learning the sequence is associated with facilitation, provides evidence that strengthens the idea that the DLPFC plays an invaluable role in sequence learning, and that the connection of DLPFC to other motor areas changes with age. However, because none of the subjects were able to recall or regenerate the sequence that was learned in our study, this would mean that the DLPFC is involved in processes guiding sequence learning without the subjects’ awareness, early during the implicit learning stage.

The role of DLPFC in motor learning is complex. There has been evidence that has argued for the DLPFC’s involvement in both explicit and implicit learning.

In a PET study by Ghilardi and colleagues, using a sequential spatial reaching task, they found significantly larger activations in the left DLPFC when subjects were required to discover the correct order of a repeating sequence by trial and error, and also when they were instructed to just remain immobile and attend to the same task without actually reaching with their hands, compared to when subjects had previously been given a sequence and had practiced this known sequence. This study is in keeping with results from our study because it shows that regional brain activation of the left DLPFC is associated with processes related to pattern or sequence searching through observing spatial cues, despite not knowing the sequence ahead of time. However, because the subjects were instructed to determine a sequence upfront, this is different from methods of our study since subjects were not advised of any sequence at all at the beginning of our task. That being said, perhaps the DLPFC is involved in a subliminal sequence searching process that translates to the results in our study; those being implicit processes in
goal-directed pattern searching that recruits activation of prefrontal networks, and particularly, the left DLPFC.

Early studies suggested that SRTT learning was impaired by low frequency rTMS over the DLPFC, but not over the SMA\textsuperscript{185}, or over the M1\textsuperscript{151}; however, some of the results have been contradictory, showing that high frequency 5Hz rTMS over M1 enhanced motor sequence learning\textsuperscript{212}, and that both ctDCS and atDCS facilitated motor sequence learning when applied during training\textsuperscript{187}. Robertson et al showed that using rTMS to disrupt DLPFC during an SRTT task would only impair learning if there was a spatial component to the visual cues, not if it there was only a colour component\textsuperscript{159}. Therefore, these studies support that the DLPFC plays a role in modulating spatial-specific information during motor learning, which is in line with the results from our study.

Furthermore, neuroimaging studies have shown that the DLPFC is activated during the fast, early learning stages\textsuperscript{11,109}, even when subjects did not have prior practice or knowledge of the sequence, and that this activation was specific to sequence learning and not non-specific learning\textsuperscript{22}. In contrast, areas such as the premotor cortex were activated in the early learning stages regardless of whether they engaged in a sequence task or a non-specific task. This is also in agreement with other studies, which also reported the DLPFC’s involvement and activation during learning of new motor sequences\textsuperscript{19}. Investigators have also postulated that the DLPFC may be involved in either encoding or acquisition of the eventual explicit knowledge of the task\textsuperscript{45}. A series of PET SRT (define SRT) studies\textsuperscript{11,19–21,26,45,178–180} showed that activity in prefrontal cortex remains high when sequence specific learning occurs. Even more convincing evidence of sequence specific learning in non-human primates have been shown in recording sequence-specific cells of the lateral PFC during production of a spatially-cued sequence\textsuperscript{181}. However, in humans, when reproducing a well-learnt sequence from memory, similar imaging studies showed no change in activity\textsuperscript{19,182–184}; rather, the production of sequence-related movements was related to increased SMA activity. The evidence from these studies point towards a dissociation in function between the prefrontal cortex and the SMA, which matches well with the external-internal theory of motor learning when discussing how the lateral versus medial motor pathways contribute to this learning.
In our study, we performed TMS as an offline-measure of how the functional connections between DLPFC and M1 change with sequence and non-sequence motor training using a pinch grip task, which showed that training did have an overall effect on the DLPFC- M1 functional connectivity, but also that the SEQ condition produced different changes than the RANDOM condition; specifically, the SEQ condition led to decreased M1 excitability, and the RANDOM condition led to increased M1 excitability. With motor training in the RANDOM condition, DLPFC-M1 excitability gradually increased with training, whereas in the SEQ condition the training caused either no change or inhibition. One idea why the different motor training conditions yielded different DLPFC-M1 connectivity results is that the DLPFC is involved in detecting the SEQ at early stages of sequence acquisition, and plays a role in providing indirect input to M1 that ultimately either increases or suppresses its excitability. When subjects learn a repeating SEQ without being aware of it, the DLPFC could be providing indirect inhibitory input to M1; when engaging in non-SEQ, generalized pinch training, the DLPFC could be providing indirect excitatory input to M1.

Furthermore, the results that show DLPFC involvement in spatial sequence acquisition is in accordance with Hikosaka’s theory to that the prefrontal cortex is involved in processing the initial sensory input and delineating a spatial sequence, before providing outputs to the associative areas of the basal ganglia and cerebellum and also eventually to the motor cortex.

An interesting observation is that the DLPFC-M1 facilitation persisted 30 minutes after the motor training; hence, the processes had at least short term effects on the DLPFC-M1 excitability. This was expected because of the existing evidence related to plastic changes in the brain that persist during online fast and slow learning, but also during off-line learning. Albert et al. used resting state imaging to examine the learning of a visuomotor tracking task, and found that over one single session, the resting functional connectivity increased in a network that involved the prefrontal, superior and inferior parietal cortices, and part of the cerebellum. Overall, this suggests that consolidation can occur after fast sequence acquisition in the frontal areas. Furthermore, Shadmerh & Holcomb showed with their PET study that during a sequence motor learning task, prefrontal areas were active during the early stages of practice in the sequence condition compared to a control condition, and this activation does not change much between the early and late learning conditions. This suggests that improvement in learning-
specific performance (in SEQ vs. RANDOM conditions) was at least in part due to the activation of the visuomotor association areas in the prefrontal cortex. The activations eventually shifted to the premotor, posterior parietal, and cerebellar cortex structures 6 hours after practice was completed. Since our experiment tested functional connectivity of DLPFC-M1 30 minutes after training, we might have captured a time when the DLPFC is still involved with motor sequence consolidation. To confirm this, it would be necessary to extend the time points for the experiments to contrast the degree of change of this functional connectivity. It would be interesting to study the effects of pinch training on the DLPFC-mediated M1 excitability over a longer training period using TMS to validate the role of the DLPFC in motor memory consolidation.

Our study results are in contrast to some of the existing evidence of the role of the DLPFC in explicit learning. We report evidence supporting the association between DLPFC and implicit sequence acquisition, since all of our subjects did not report noticing a sequence after completing the task. Wilkinson et al.\textsuperscript{186} showed in their continuous theta burst TMS study that applying an inhibitory type of repetitive TMS, termed continuous theta burst stimulation (cTBS) to M1 improved the early stages of motor learning, while stimulating the DLPFC with inhibitory cTBS had improved learning in the later stages of the task in a modified probabilistic serial RT task. These findings suggest that inhibition of the DLPFC has an effect on explicit motor learning, and that perhaps the DLPFC plays a role in explicit learning. With the current mix of evidence showing the involvement of the DLPFC in both explicit and implicit learning, the difference in results could be due to the differences in study design. However, the DLPFC could indeed play a role in both implicit and explicit aspects of learning: “implicit” in the sense of learning where the subject is unaware that a sequence existed, and “explicit” in the sense that subjects begin the task expecting a sequence. The previously cited studies fit either of these two criteria—perhaps the DLPFC’s involvement in sequence acquisition in early learning has more to do with pattern searching, be it at a conscious or subconscious level—and not so much with actually recalling a well learned sequence or pattern. Studies have shown that in a random sequence of binary events, where one alternative occurs more often than the other, humans tend to try to guess which alternative is correct based on previous occurrences\textsuperscript{214,215}. A tDCS study by Hecht et al.\textsuperscript{216} showed that participants became quicker at selecting the most frequent alternative when anodal tDCS was applied to the left DLPFC, adding evidence of involvement of this area in probabilistic
learning and reasoning\textsuperscript{217,218}. Therefore, this leads for us to suggest that perhaps the left DLPFC is involved with searching for a spatial pattern based on what subjects experienced in previous trials, and that this process may be active without subject awareness.

**Implicit Sequence Acquisition after Motor Pinch Task: The Importance of M1**

Our measures of M1 excitability indicate that motor training in general increases excitability 30 minutes after training was completed, consistent with the notion that motor training to the specified muscle induces use-dependent plasticity. A hallmark experiment by Karni et al (1995)\textsuperscript{219} showed functional MRI evidence for the adult motor cortex plasticity during motor skill learning, and since then a body of evidence suggesting that training-induced plasticity in the motor area occurs has been accumulated and become widely accepted. Motor training did not increase excitability of M1 during our training task and even immediately after training was completed; there was actually inhibition in the SEQ visit in the after the second block of motor training and this inhibition was significantly different from the level of excitability in the RANDOM visit. A possible explanation for the decreased excitability of M1 during and immediately after the pinch training task in the sequence condition could be that M1 activity needs to be suppressed during the global efforts of the brain to process and acquire the sequence. The “During” TMS sampling stage took place after the second block of training. There is a possibility that other areas of the brain are more active during the early stages of sequence acquisition the sequence, and this activity competes with and leads to a temporary suppression of M1 excitability. Interestingly, the suppression of M1 excitability at the “during” stage coincides with the increased skill measure at block 3 of training for both SEQ and RANDOM visits, though this does not imply a causation.

**Pinch Task Skill Measure**

Our task was adapted from Reis et al’s 2009\textsuperscript{33} experiment, where they measured skill acquisition over 5 days and we measured skill acquisition in a cross-over design. From Reis et al’s learning curve data, most of the improvements in the training task were reaped within the first day of training; hence our paradigm adapts a similar task, but over a training period of one day since we were more interested in looking at the overall differences between sequence specific training and non-sequence motor training, rather than accumulated training benefits. For our experiments,
over the ten training blocks for both SEQ and RANDOM visits, there was an effect of training on the skill measure, and when tested at block 9, subjects in the SEQ visit experienced a significant decrease in skill measure, suggesting that these subjects unknowingly learned the sequence (see Figure 19).

The sensitivity of subjects to the randomized sequence is a critical measure to test whether they demonstrated sequence acquisition through behavioural measures during the course of that training task. The serial reaction time task uses a similar paradigm in its task: a robust task where subjects use fingers to press buttons in response to stimuli on a screen\textsuperscript{12}, and the outcome measures are typically accuracy and reaction time. The SVIPT differs compared to the SRTT in that there is an added force component to the task, and hence an increase in attentional demand; subjects are required to “pinch” their thumb and phalanges against a force transducer to reach the cued targets. Through careful instruction, we ensured that the main muscle that being used was the first dorsal interosseus muscle, since the pinch movement in the hand activates this muscle predominantly. We hypothesized that through localizing the muscle of interest to train in our task, we would be able to observe the neurophysiological effects of motor training, and of sequence acquisition, in the FDI muscle recordings later with TMS. Practicing motor tasks with a higher degree of processing difficulty are more likely to be associated with motor cortical change, compared to non-skilled or simple motor behaviour\textsuperscript{220,221}. Through using a variation of the SRTT, we were also able to collect more dimensions and outcome measures of sequence acquisition in the pinch task, such as MT and spatial error.

**Clinical Applications**

Learning more about the DLPFC’s role in sequence acquisition has important implications for the clinical population. Particularly, achieving experimental results that either coincide with or disagree with previous work can assist us in determining the involvement of DLPFC in various diseases that affect cognitive domains, such as stroke and movement disorders such as Parkinson’s disease. Determining exactly how the DLPFC functions can help guide the development of feasible and effective approaches towards rehabilitation and treatment.

Mentis et al\textsuperscript{222} studied early stage, non-demented Parkinson’s disease patients and normal volunteers using partial least squares analysis in PET imaging and compared their respective
mechanisms of sequence learning. The left DLPFC was activated in a large area (extending from 6 mm to 38 mm relative to the anterior commissure-posterior commissure line), early in sequence learning in healthy volunteers, which supports the results of previous neuroimaging studies. Their study concluded that in order for PD patients to achieve the same degree of performance as healthy controls in a task with low difficulty (not normally causing bilateral activation in healthy controls), patients recruited large bilateral networks normally specialized for sequence learning, and that this bilateral information processing is different from and less efficient than the information processing in healthy controls. Our own correlational results between age and DLPFC-M1 functional connectivity show a moderate negative relationship, suggesting that older subjects experience more DLPFC-mediated M1 inhibition than younger subjects. This could support the notion that disruptions or changes in integrity to the prefrontal area, whether from old age or from neurological diseases, can change the resulting functional input of this area to the primary motor cortex, and this subsequently could have an effect on the behavioural measures of sequence acquisition.

A stroke fMRI study by Meehan et al.\textsuperscript{223} showed that stroke patients with right focal subcortical lesions displayed intact implicit motor learning compared to healthy controls in a joystick motor training task (comparing a sequence motion to a generalized, non-specific motion in their right hands). This study showed that in healthy subjects, sequence learning led to increased BOLD signal in the left dorsal premotor cortex, whereas the stroke patients exhibited increased BOLD signal to the left DLPFC. This study supports the role of DLPFC in implicit motor learning after stroke. Furthermore, Gomez-Beldarrain et al.\textsuperscript{224,225} provided evidence that the prefrontal cortex is involved with learning aspects of particular action plans, rather than generalized features. They studied patients who had prefrontal lesions, who were impaired in a tracking task that involved learning specific movement sequences, but were unimpaired when the movement was non-specific. Interestingly, sleep improved sequential motor learning performance in patients with prefrontal lesions.\textsuperscript{226}

Other behavioural outcome measures also showed that motor sequence acquisition had occurred during the training task. MT decreased with motor training, indicating that the subjects reached the targets and returned to the HOME position faster with each training block, and this was consistent for both the SEQ and RANDOM visits. There was no effect of condition on the MT.
The results of spatial accuracy over the ten training blocks also did not show evidence of sequence acquisition. It is reported in previous studies that motor sequence acquisition is often captured in some outcome measures, but not other outcome measures in a particular training task, and this could be due to various factors. For instance, in this study, subjects were asked to reach the targets as quickly and as accurately as possible, with a “hit” being defined as touching any part of the target with the cursor. Since subjects were not asked to try to hit the center of the target as closely as possible, it was not surprising that the spatial accuracy results did not yield values that would be consistent with improvement in this measure.

**Limitations**

Since our subjects came either in the morning or the afternoon for their experiments, one possible limitation is the confounding factor of time of day that may influence some of the results. Previous studies have examined this issue, looking at the effects of sleep on corticospinal and intracortical excitability in the human motor system. Doeltgen & Ridding\textsuperscript{227} showed that SICI, ICF, and input-output curves did not change in the morning vs. the afternoon on the same day, adding evidence that motor cortical excitability measures are not strongly influenced by time-of-day dependent variability, and therefore is unlikely to be a confound in our study.

Secondly, the stimulation current spread of TMS is also another limiting factor of the study. Previous studies have suggested that the effects of TMS, and particularly rTMS, may spread along neuronal connections and influence distant subcortical and cortical substrates\textsuperscript{228,229}. The spread of TMS current directly to brain tissue is thought to be limited in area, and is influenced by stimulation intensity, frequency, and duration. Mapping studies using a small figure-of-eight coil have a spatial resolution of roughly 0.5-1cm\textsuperscript{230,231}; however, other studies have argued that this spatial resolution is misleading because the extent of TMS motor output maps are mainly determined by current spread, and by the relationship between the coil position on the scalp and the depth of the motor output region in the cortex\textsuperscript{232}. On the other hand, further studies suggest that TMS is comparable to PET, fMRI, or direct cortical stimulation experiments in terms of spatial resolution, focality, and specificity of the effects\textsuperscript{233–236}.

Thirdly, another factor that is related to the focality of TMS is the inter-subject variability in markings of stimulation sites for the TMS coil placement. The purpose of TMS is to stimulate a
specific anatomical brain area through applying a current from the coil through a subject’s scalp. However, brain-scalp relationships are different across individuals, and using landmarks to determine coil placement would introduce errors and variability\textsuperscript{237}. Instead, using a standardized measurement from the area of the motor cortex that elicits the largest MEP output in a particular contralateral hand muscle is a more reliable method\textsuperscript{238}. Nonetheless, there is still error associated with this method, due to variability in brain size and anatomy; for example, using a standardized 5 cm measurement anterior from the FDI muscle motor hotspot as the DLPFC would be problematic if a subject’s head is much larger or smaller than the average.

The MNI co-ordinates for the DLPFC in our study showed considerable variability, which is a potential limitation to our study. We were only able to obtain the co-ordinates of DLPFC stimulation for 10 subjects, and the large range indicates that not all subjects were stimulated in the same area of DLPFC. This could potentially confound our results, if sub-regions of the DLPFC are involved in different functions. DLPFC is a large functional area. Our data suggests that the areas we stimulated for the study are within the functional area, but there is room for improvement to minimize variability. More accurate approaches can be taken, such as online neuronavigation during TMS as opposed to after TMS. For our study, some subjects had their MRI scans between their two visits, and some subjects did not have scans. Thus, we could not use the online approach. In the future, online head-surface digitization and registration of TMS stimulation sites for every single subject’s 3-D reconstructed head MRI can help address the issue of anatomical specificity.

A fourth limitation of our study is that we cannot guarantee that subjects were not mentally rehearsing the task during the TMS. Although they were instructed to remain alert, not to think about movement, and maintain their muscles relaxed during stimulation, we cannot exclude the possibility that some subjects could have been thinking about the task, which could subsequently affect the TMS measures. It has been shown that mental rehearsal and imagery can change corticospinal excitability\textsuperscript{254}.

Another limitation of our study involves subject enrolment. It would have been optimal for us to have a larger population to study that were middle-aged (between 40-60 years old) to add to the correlation analysis. However, we were limited to subjects who volunteered for the study and
qualified for it after their screening and neurological examinations. Thus, a future direction involves recruiting more subjects from this age group. Furthermore, another approach at age analysis could also involve grouping subjects into “young” (under 30 years of age) versus “old” (older than 60 years of age), as has been done in previous studies on the effects of age. With a larger study cohort, we will be able to perform variations in the statistical analysis to confirm our results of age-related effects on the behavioural and electrophysiological TMS measures.

Finally, the results obtained in this study could be strengthened if we had included an additional group or visit for subjects where a different site was stimulated, to show that our results were indeed site-specific; this is a possible avenue to pursue in future studies related to the role of the DLPFC in motor sequence acquisition.
CHAPTER 6: CONCLUSIONS/FUTURE DIRECTIONS

In future studies, we would like to build upon the results from this study by studying this DLPFC-M1 connectivity over a longer period of time, i.e. days or months, to examine the stability of this increased connectivity due to sequence acquisition. This will allow us to examine motor skill learning, wherein perhaps the plastic brain will incorporate permanent changes in its networks and circuitry after acquiring this skill for the long-term.

In addition, in future sequence learning studies, we can test at the subjects’ ability to “recall” a sequence—for instance, if we provide a multiple choice test with sample sequences to test their ability to pick out the correct sequence during the pinch task. This will give us more information regarding what stage the subjects are at for acquiring the sequence knowledge. Furthermore, we would like to study this connectivity in patients with Parkinson’s disease or stroke as a next step, to look at how the DLPFC-M1 connectivity changes in neurological diseases. If we find that this connectivity is different in patients than in healthy subjects, the next step would be to devise non-invasive techniques to modify the activity of DLPFC, such as rTMS or paired-associative stimulation (PAS) to ameliorate this deficit in DLPFC-M1 connection and restore impairments in goal-directed behaviour and sequence learning. Finally, increasing the pool of subjects would increase the power of our study, and adding additional controls studies (i.e. a control TMS stimulation site, recording from muscles not involved in the pinch task) would yield even more convincing results in future studies.

In conclusion, our results on healthy subjects across the age span suggests that (1) age is related to changes in functional connectivity of the DLPFC to M1; (2) acquiring the sequence (as shown in testing block 9 for RT, spatial accuracy, and Accuracy/RT skill measure) during the motor pinch training task is related to increased facilitation in the DLPFC-M1 connection. We provided evidence that DLPFC plays a role in implicit sequence acquisition that manifests both through our DLPFC-M1 dual site TMS measure and also through correlations between behavioural and electrophysiological results. These results add to the existing motor sequence learning literature by providing, for the first time, evidence of changes in functional-connectivity between DLPFC and M1 during motor sequence acquisition. These study results provide insight on the effects of
age and motor sequence acquisition on the resulting functional connection between DLPFC and M1, and these results can bridge translation to studies in the near future for stroke patients and patients with movement disorders through contributing to rehabilitation strategies.

Contributions

I would like to thank my supervisor, Dr. Robert Chen, and my committee members Dr. Antonio Strafella and Dr. Timothy Welsh, for their expertise and astute suggestions for my project; Dr. Michael M. Vesia for offering great advice and being a great mentor throughout this project; Carolyn Gunraj for her constant insight and help in executing the TMS experiments; Ayda Ghahremani for her knowledgeable expertise in MATLAB data analysis and statistical analysis; Gaayathiri Jegatheeswaran for assisting in the Brainsight neuronavigation technique; Kit Beyer for his advice and contributions towards the Labview program and towards the design of my experimental apparatus; and finally, our summer student Jeanette Hui for assisting in coil holding during experiments.
References


doi:10.1007/BF00231050


doi:10.1007/BF00232190


42. Scahill, RI, Frost, C & Jenkins, R. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Archives of* ... (2003). doi:10.1001/archneur.60.7.989


89. Stoney, SD & Thompson, WD. Excitation of pyramidal tract cells by intracortical microstimulation: effective extent of stimulating current. *Journal of ...* (1968).


doi:10.1093/brain/122.5.855


doi:10.1002/ana.410380611

doi:10.1126/science.8122113


doi:10.1212/WNL.48.5.1398


166. Pogarell, O, Koch, W, Pöpperl, G & Tatsch, K. Acute prefrontal rTMS increases striatal dopamine to a similar degree as D-amphetamine. Psychiatry Research: ... (2007).


187. Eliassen, JC, Souza, T & Sanes, JN. Human brain activation accompanying explicitly
doi:10.1007/s002210100822

188. Barone, P & Joseph, JP. Prefrontal cortex and spatial sequencing in macaque monkey.

doi:10.1093/brain/121.2.253

190. Deiber, MP, Ibañez, V & Sadato, N. Cerebral structures participating in motor

191. Sadato, N, Campbell, G & Ibañez, V. Complexity affects regional cerebral blood flow

stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. *The Lancet* 348,

Primary Motor Cortex is Essential for Probabilistic Implicit Sequence Learning: Evidence from
doi:10.1162/jocn.2009.21208

194. Nitsche, M. *et al.* Facilitation of Implicit Motor Learning by Weak Transcranial Direct
Current Stimulation of the Primary Motor Cortex in the Human. *Journal of Cognitive

*Neuropsychologia* (1971).


199. George, MS, Wassermann, EM & Williams, WA. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. ... (1995). doi:10.1097/00001756-199510020-00008


235. Parsons, LM & Osherson, D. New evidence for distinct right and left brain systems for

236. Ungerleider, LG, Doyon, J & Karni, A. Imaging brain plasticity during motor skill

*Exercise and sport sciences* ... (2005). doi:10.1097/00003677-200501000-00005

238. Plautz, EJ, Milliken, GW & Nudo, RJ. Effects of repetitive motor training on
movement representations in adult squirrel monkeys: role of use versus learning. *Neurobiology
of learning and memory* (2000).

patients and normal volunteers: comparative mechanisms of sequence learning. *Human brain

learning after subcortical stroke is associated with increased prefrontal brain activations: An

241. Beldarrain, MG, Gafman, J & de Velasco, IR. Prefrontal lesions impair the implicit and

242. Beldarrain, MG, Grafman, J & Pascual-Leone, A. Procedural learning is impaired in

243. Beldarrain, MG, Astorgano, AG & Gonzalez, AB. Sleep improves sequential motor
learning and performance in patients with prefrontal lobe lesions. *Clinical neurology and
...* (2008).

244. Doeltgen, SH & Ridding, MC. Behavioural exposure and sleep do not modify
corticospinal and intracortical excitability in the human motor system. *Clinical Neurophysiology*
(2010).


doi:10.1152/jn.00386.2014