

**DOES SHORT TERM UPPER LIMB IMMOBILIZATION AFFECT INTER-LIMB  
TRANSFER OF SEQUENCE-SPECIFIC MOTOR SKILL?**

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TRANSFER OF SEQUENCE SPECIFIC MOTOR SKILL?**

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## ABSTRACT

Previous research has demonstrated that short-term limb immobilization leads to a decrease in corticospinal excitability and a subsequent increase in the capacity for long term potentiation, the neural mechanism underlying learning. Whether immobilization directly leads to the enhancement of motor learning is currently unknown. Moreover, the effect of short-term immobilization on the inter-limb transfer of motor skill has not been quantified. Interlimb transfer refers to skill improvement in one limb being followed by skill gain in the opposite, untrained limb providing an index of skill generalization to the untrained limb. Understanding inter-limb skill transfer could lead to enhanced motor skill learning in health and disease. The present study aims to train an implicit sequence on the left hand and assess motor skill performance in the untrained, right hand of individuals at baseline (AM) and during a follow-up assessment (PM). Thirty-six young, healthy participants were randomly assigned to a motor skill training condition (left hand trained or left hand untrained) in the morning (AM) followed by an immobilization condition (immobilized or non-immobilized between sessions) then follow-up testing in the evening (PM). Trained participants completed a single training session using a modified serial reaction time task containing an embedded repeating sequence. Untrained participants completed short skill test blocks before the immobilization period but did not undergo the left-hand training paradigm in the morning. In all participants, general motor performance and sequence-specific performance were compared in the AM and PM sessions. Results showed AM to PM improvement in general skill performance in the Trained-Immobilized group ( $p=0.045$ ) but a decrease in sequence-specific task performance in all groups. It can be suggested that immobilization enhanced the capacity of general motor learning and led to the inter-limb transfer of *non*-sequence-specific general motor skill.

## INTRODUCTION

Neural plasticity is the capability of the central nervous system to adapt and change functionally and structurally and underpins learning and recovery after a stroke. Use-dependent plasticity, or the change that occurs in a particular brain area when engaged in a specific behavior, underlies motor learning and motor memory formation<sup>1</sup>. Specifically, repetitive and prolonged movement induces neural plasticity and subsequent improvements in task performance<sup>2</sup>. While research has exhibited a link between plasticity and motor learning, the extent to which neural plasticity can be harnessed to optimize learning warrants continued exploration.

Learning a motor task, such as riding a bike or texting on a phone, involves the formation of a memory. Explicit memory differs from implicit memory in that it relies on the conscious retrieval of information, while implicit memory does not depend on conscious recollection of previous experiences.<sup>3</sup> In the context of learning, explicit learning involves an awareness of the underlying goal(s) of learning, while implicit learning is absent of the awareness.<sup>4</sup> Both types of learning are present in our daily lives—formation of biases often involves implicit learning while memorizing the 206 bones in the human body involves explicit learning. Memory formation involves essential processes—encoding, storage/consolidation, retrieval and reconsolidation.<sup>5</sup> Encoding is the initial input and processing of information. Consolidation involves the storage and maintenance of information over a period of time, and retrieval is the capability to access stored information. Memory reconsolidation is the distinct process of maintaining and modifying stored memory traces.<sup>6</sup> Skill learning requires memory formation; whether it be of explicit or implicit nature is dependent on if an individual is aware of the information being acquired.

Motor learning is the improvement of motor skill beyond baseline performance that leads to a sustained decrease in performance error over time<sup>7</sup>. Motor learning involves both sensory and motor components, as sensory feedback is required to learn skill with minimal errors<sup>8</sup>. Plasticity in the primary motor cortex (M1) is important in learning of trained movements. A previous study in rodents demonstrated that practicing paw maneuvers to retrieve a food reward led to structural changes in the forelimb representation of primary motor cortex<sup>9</sup>. In a proprioceptive discrimination task, short-term restructuring of the sensorimotor cortex correlated with participants' ability to learn a motor task, suggesting that neural plasticity is associated with the motor learning of the task<sup>10</sup>. It is thought that neural plasticity correlates with the capacity to

acquire skilled movements. It is of interest to investigate ways in which the brain's plastic abilities can be modulated to enhance motor skill acquisition.

Implicit sequence learning has been studied in literature with the serial reaction time task (SRTT)<sup>11,12,13,14</sup>. The SRTT instructs participants to indicate the location of a temporary flashed stimulus by pressing the button that corresponds to the location, while maintaining speed and accuracy to complete the task. The stimuli are presented as blocks of a fixed number of sequenced stimuli. Through repeated presentation of sequence blocks, a participant's average response time to the flashed stimulus should decrease due to consistent training, while, in contrast, average response time should increase when the sequence block is interrupted.<sup>15</sup> Performance during the SRTT is primarily mediated by general motor learning but it also has influence from implicit perceptual-based learning, such as the prediction of the next stimuli in the stimulus presentation, if sequences are presented in a pattern<sup>16</sup>. The SRTT is a useful paradigm to induce motor skill learning, while also measuring individuals' trajectory of learning using response time data.

The consolidation of the sequence patterns presented in the SRTT are of interest in motor task learning. "Offline" memory consolidation is the period after task acquisition that is suggested to enhance procedural memory<sup>17</sup>, a form of implicit memory. In an experiment utilizing an explicit task, two distinct forms of learning—sequence-specific learning and general motor skill learning—were demonstrated during offline consolidation of the task<sup>18</sup>. While sequence-specific learning describes the acquisition of a sequence-specific pattern<sup>19</sup>, the latter describes improvements (i.e. reduction in response time) in motor performance over a sustained period of time and is demonstrated when pressing random and un-sequenced buttons. An explicit task involves participants being aware and conscious of the task's goal(s). Both sequence-specific learning and general motor performance were seen to improve for explicit tasks during offline consolidation.<sup>18</sup> However, such results are not present with an implicit task, or a task that involves completion without awareness of the task's goal. In a study using follow-up assessments 24 hours and one-week after SRTT training, it was demonstrated that offline consolidation led to improvement in general motor skill memory but no augmentation of sequence-specific memory with either interval.<sup>19</sup> Given the varying results with an implicit task, it will be interesting to compare sequence-specific task performance with changes in general motor skill performance in the present study. Studies have related the consolidation of motor

sequence learning to plastic changes in the brain<sup>20,21</sup>. Furthermore, there is evidence that the acquisition of skill during implicit sequence learning is associated with functional restructuring of the corticospinal tract<sup>22</sup>. Plasticity and its relation to motor skill consolidation is of importance to the present study. It will be studied whether implicit acquisition and offline consolidation of sequence-specific motor skill is observed after a six-hour immobilization period.

Previous studies have used short-term sensorimotor deprivation to study its effect on corticospinal excitability. Corticospinal excitability is facilitated by the corticospinal tract, the white matter pathway that conducts impulses from the activated motor cortex to motor neurons executing the action. Short-term limb immobilization, or the temporary dis-use of a limb, has been demonstrated to reduce corticospinal excitability, as it causes a reduction in sensory input and motor output<sup>27,28</sup>. The mechanism that underlies the excitability change and whether it translates to neural plasticity was investigated. Experiments indicated that a decrease in the corticospinal excitability to the target muscle was accompanied by an increase in long term potentiation (LTP)-like plasticity<sup>29</sup>. LTP is the neural mechanism that underlies the strengthening of synapses and leads to learning and memory formation. With immobilization's suggested effect on LTP and the induction of neuroplasticity, it is conceivable that learning could be affected as well. Studies have explored the effects of immobilization on the behavior of the immobilized limb; however, current literature lacks understanding of what changes—both in behavior and in neurophysiology—occur in the contralateral, non-immobilized limb.

Training one limb has shown improvements in the opposite limb. Several studies have observed task performance improvement in the trained limb and a performance gain in the nontrained limb<sup>23, 24, 25</sup> attributed to the transfer of skill. This inter-limb skill transfer is thought to be attributed to changes in bilateral corticospinal excitability<sup>25, 26</sup> and the release of intracortical inhibition<sup>24</sup>. Whether limb-transfer is affected by sensorimotor deprivation is of interest; it is currently unknown whether the degree of use of the trained limb influences the level of skill transfer to the opposite limb. Further investigation into skill performance in the untrained limb can lead to a better understanding of the transfer phenomenon.

While it has been suggested that short-term immobilization enhances the capacity for plasticity, it is unknown whether this enhancement leads to improved skill learning in both the trained and untrained limb. I hypothesize that short-term immobilization of the trained arm will prompt a heightened plastic capacity that augments pattern-sequence skill acquisition in both the

trained and untrained limb following the immobilization period. Specifically, I propose that short-term left arm immobilization will facilitate the transfer of sequence specific skill from the trained, left hand to the untrained, right hand. Ultimately, the understanding of the short-term immobilization effect on modulation of neuroplasticity and motor performance can provide an understanding on how to enhance learning in an untrained (perhaps impaired) limb. Such a model can aid in better understanding and augmenting motor rehabilitation after a neural injury, such as stroke.

## **LITERATURE REVIEW**

The concept of altering neural plasticity to understand motor function, and more recently, motor learning, has been studied in depth in the last decade. The involvement of neurotransmitters and their receptors, such as gamma-aminobutyric acid (GABA), in neural plasticity has been extensively studied. Plasticity, or the adaptive and remodeling capacity of the brain, has been of interest to the injured brain. My study, as part of the Neural Plasticity Research Laboratory (NPRL), aims to understand the plastic nature of the brain and its capabilities to improve task acquisition after sensorimotor deprivation and transfer acquired skill to an untrained limb. The study's findings aim to inform the development of motor learning, eventually improving rehabilitation efforts for individuals who have experienced major brain trauma, such as stroke.

Neural plasticity is the brain's ability to reorganize itself, structurally and functionally. Plasticity has been largely attributed to use<sup>2,9,30</sup>. Butefisch et al. (2000) related the concept of use-dependent plasticity (UDP) with synaptic manipulation by using a drug to manipulate neurotransmitter receptors to influence plasticity in neuronal synapses. The drug manipulation was observed to enhance GABA inhibition and block NMDA receptor activation, leading to a substantial decrease in use-dependent plasticity.<sup>31</sup> Butefisch et al. (2000) suggested that a decrease in GABA levels facilitates long term potentiation (LTP) in the motor cortex. These results show that a downregulation of GABA levels is necessary for motor skill learning. The concept of use-dependent plasticity and its underlying of motor learning is of important relevance to the current study, especially as it relates to temporary disuse of a limb, or limb immobilization. The relation of immobilization with UDP can be studied in the context of motor skill learning, such as with the serial reaction time task (SRTT).

The serial reaction time task (SRTT) is a task that was developed in 1987 by Nissen and Bullemer<sup>32</sup>. The SRTT has been used to study motor skill learning in healthy and diseased or injured brains. Motor skill learning can be induced by the SRTT,<sup>11</sup> and whether the type of motor learning is primarily perception based or motor based was investigated<sup>17</sup>. Perception-based learning is the learning of the stimulus sequence such that a predictive sequence element causes a decrease in reaction time<sup>33</sup>. While the former is stimulus-stimulus learning, motor-based learning is an effector muscle's response to motor information sequences, or response-response learning<sup>33</sup>. It was suggested that while perceptual learning plays a salient role in implicit learning, it is ultimately motor-based learning that induces implicit learning in the SRTT. This finding from Kemeny & Lukas (2011) is consistent with earlier work done by Willingham et al. (2000) that indicated implicit learning is primarily motor based<sup>34</sup>. Meier & Cock (2014) examined implicit learning beyond the acquisition period, to an offline consolidation period where no further practice of the skill was performed. It was suggested that during an offline period after the SRTT, general motor learning of an implicit pattern was enhanced, while sequence-specific learning did not undergo offline memory consolidation<sup>19</sup>. Such a result is seen in implicit learning. In explicit learning, there was an improvement in both sequence-specific learning and general motor performance during the offline consolidation period.<sup>19</sup> Using the SRTT, an implicit sequence task will be trained on the left hand, but performance measures will be analyzed from the un-trained right hand following a six-hour offline consolidation period.

The effect of the immobilization period on the inter-limb transfer of acquired sequence-specific skill is the central investigation in the study. Through sensorimotor deprivation, immobilization is suggested to have differential effects on corticospinal excitability depending on the duration of the immobilization. A reduction in corticospinal excitability was observed in immobilization periods of eight<sup>29</sup> to four days<sup>35</sup>, while an increase of excitability was observed in longer immobilization periods, such as 30 days<sup>36</sup>. During the eight-hour immobilization period, it was suggested that the activation of homeostatic plasticity was the underlying mechanism behind the reduction in corticospinal excitability.<sup>29</sup> With decreased corticospinal excitability leading to enhanced LTP-like plasticity<sup>29</sup>, an immobilization period of eight hours may be a short enough duration to induce learning, while maintaining feasibility with participants. Limb immobilization has been related to the induction of slow wave activity (SWA), which has been linked to an increase in cortical plasticity<sup>27</sup>. While the induction of plasticity has been suggested

with an immobilization period<sup>27,28,29</sup>, whether it leads to enhanced motor skill consolidation is yet to be investigated.

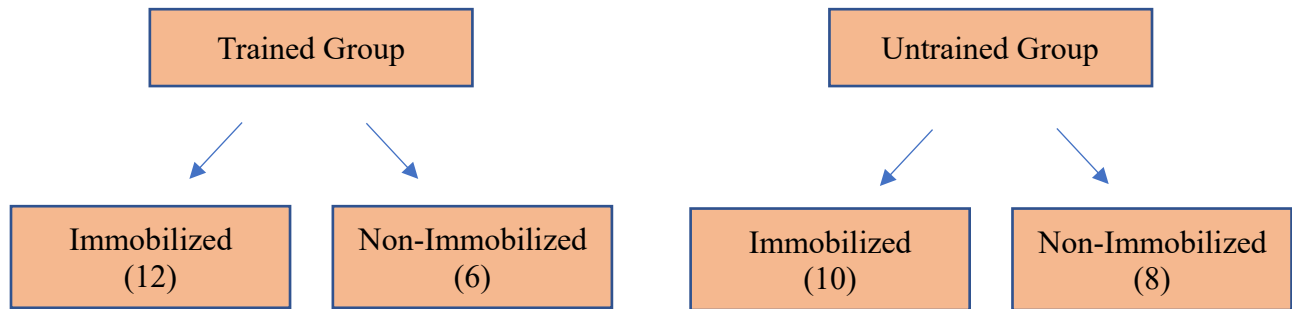
By studying the behavioral data from the SRTT, patterns of task performance across sessions and between the immobilized and non-immobilized group will be compared. The present study will investigate the effect of sensorimotor deprivation on motor skill learning in healthy, young individuals; moreover, it will study whether immobilization results in greater inter-limb transfer of sequence-specific skill to the untrained hand. With an understanding of immobilization's effect on inter-limb transfer, limb rehabilitation efforts can aid in skill improvement of the paretic limb by utilizing motor learning in the opposite, fully functional hand. In this way, motor skill learning can potentially be optimized in both limbs through inter-limb skill transfer.

## **METHODS**

The present study aimed to investigate whether an inter-limb facilitatory transfer effect can be induced on right hand task performance from skill training and immobilization of the opposite (left) hand.

### ***Group Assignment***

The effect of a six-hour left arm immobilization period on behavioral measures was assessed using a motor learning serial reaction time task (SRTT). Forty healthy, right-handed individuals from the age of 18 – 35 years participated in data collection. Data from four individuals were excluded due to time constraints, abnormally fast and expert-like motor performance, or misinterpretation of task instructions. Participants were randomly assigned into one of four groups: Immobilized and Trained (N=12), Non-Immobilized and Trained (N=6), Immobilized and Untrained (N=10), and Non-Immobilized and Untrained (N =8) (figure 1). The Trained group underwent left hand task training in the morning, and the Untrained group underwent left hand training in the afternoon. The Untrained group was considered the control group, since the six-hour immobilization period was not preceded with task training. It was expected that offline task consolidation would only occur in the Trained group.



**Figure 1. Randomized Group Assignments.** Participants were first randomly divided into two groups—trained and untrained. “Trained” represents individuals who underwent left hand training prior to the six-hour immobilization period. The “Untrained” group consists of individuals who completed left hand task training in the afternoon session. From there, participants were randomly divided and assigned to an immobilization state.

### *Arm Immobilization Procedure*

Participants were randomly assigned to be in either the Immobilization group or Non-Immobilized control group. Those assigned to be immobilized wore a finger control mitt over the left, non-dominant arm, with a sling over the mitt to hold the arm in place (figure 5), for six hours starting after the conclusion of the AM session. All participants, including those not immobilized, wore an activity monitor (wGT3x-BT, ActiGraph) on the both wrists to ensure immobilization compliance and to monitor bilateral arm activity.

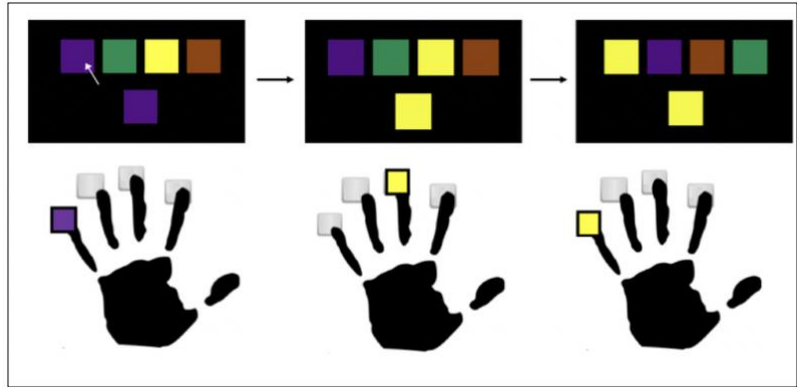


**Figure 2. Immobilization arrangement.** The mitt controls for finger movement, while the sling reduces wrist and elbow movement.

### *Motor Learning Task*

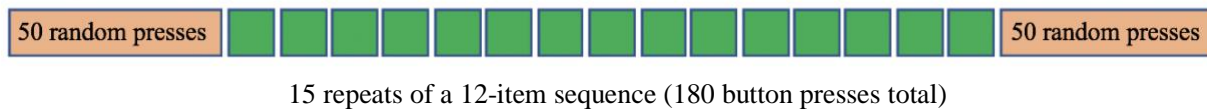
In the motor task, the left hand was trained on a modified version of the SRTT. The SRTT requires participants to press a button that corresponds to a visual stimulus on a screen directly in front of them (figure 2). The four squares in the top row correspond to the four buttons on the button box, and the participant was asked to press the button that matched the target (bottom) square. The target square stimulus appeared as one of the four colors of the four

squares: green, brown, purple, yellow. Once a participant selected the correct button, the screen changed to a novel array of the four colored squares and a novel target stimulus. While the participant was aware to complete a color-matching task, the sequence of button presses was intended to be learned implicitly.



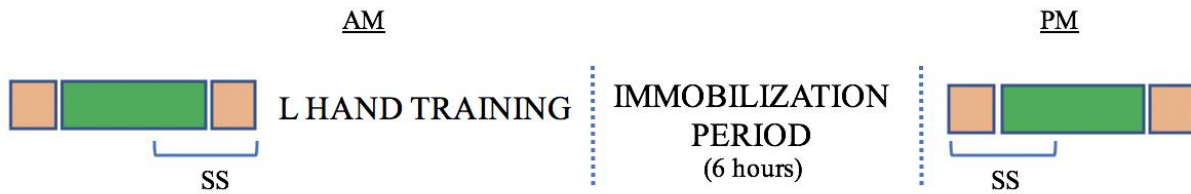
*Figure 3. Serial Reaction Time Task (SRTT) Illustration. Individuals match the bottom target square to a novel array of 4 colored squares presented on a screen. Participants will press the button that corresponds to the placement of the colored square on the top row of the screen. The target stimulus and order of 4 top squares will change, and the task will continue for 180 presses in training, or 280 presses in the test block.*

The training period consisted of five rounds of 180 sequenced button presses. The 180 button presses included 15 consecutive repeats of a 12-item sequence. Each block of a 12-item sequence was referred to as a “repeated block”. The effect of left-hand training was measured by test blocks in both the left and right hand. Skill assessments were performed during both sessions via test blocks. The test blocks consisted of 180 sequenced button presses with 50 random button presses at the beginning and end of the block, totaling to 280 presses (figure 3). Test blocks were performed before (Pre) and after (Post) sequence training. The participants in the Trained group completed an additional retention (“Ret”) test block after the immobilization period in the PM session. In contrast, the participants training in the evening, or the “Untrained” group, performed one “AM Pre” test block in the morning and two in the evening—“PM Pre” and “Post”. Although a total of three test blocks were performed in both the Trained and Untrained group, only the two test blocks flanking the immobilization period were analyzed (figure 4). The response time during the “Post” test block and “Ret” test block were compared in the Trained group (figure 4a) while the “AM Pre” test block was compared with the “PM Pre” test block in the Untrained group (figure 4b).



*Figure 4. Test block. Consists of 50 random button presses flanking the repetitive set of 180 button presses, with a total of 280 button presses. It is presented before and after training, as well as after the immobilization period.*

a) Trained Group Data Collection Overview



b) Untrained Group Data Collection Overview



**Figure 5. Setup of Testing Sessions Dependent on Timing of Left-Hand Training.** Pictured in green and orange are the test blocks of interest, with orange representing 50 random buttons and green representing the 180-button sequence. Brackets indicate where in the test block Skill Score is calculated (flanking the immobilization period). **4a.** Schematic specific to the Trained group that underwent left-hand training prior to the immobilization period. **4b.** Schematic specific to the Untrained group that did not undergo left hand training prior to the immobilization period (control group).

### ***Skill Score Calculation***

A “Skill Score” (SS) was calculated to isolate and compare the performance on the sequence button presses from the performance on the random presses. Skill Scores were calculated in both the AM and PM session. The AM SS was calculated by subtracting the mean response time during the last 50 random presses with the last four repeated sequence blocks in the test block. In contrast, the PM SS compared the response times of the first 50 random button presses with the first four repeated sequence blocks. The PM SS compares performance in the beginning of the test block in order to quantify a potential immobilization effect and to control for any possible performance improvement with increased exposure/practice in the afternoon.

While training occurred between the two skill assessments in the Trained group, training did not occur between the skill assessments in the Untrained group (figure 4). The Untrained group acted as a control for circadian fluctuations in motor performance. A Skill Score of approximately 0 is expected prior to training, as the performance of sequenced presses and random presses should be equivalent in speed. In contrast, a positive SS is expected after training, as sequence performance should improve with training.

### ***Change Score Calculation***

Change Score (CS) is a metric used to analyze AM to PM changes in performance with random button presses (general motor performance) and sequenced button presses (sequence performance). Change Score was calculated by subtracting AM average response time data from PM average response time data. A reduction in PM average response time results in a negative CS and indicates performance improvement. General motor performance CS was calculated by comparing response times of the 50 random button presses in the test blocks. Sequence performance CS was calculated by comparing the average response times of the last four rep blocks in the AM session with the average response time of the first four rep blocks in the PM session.

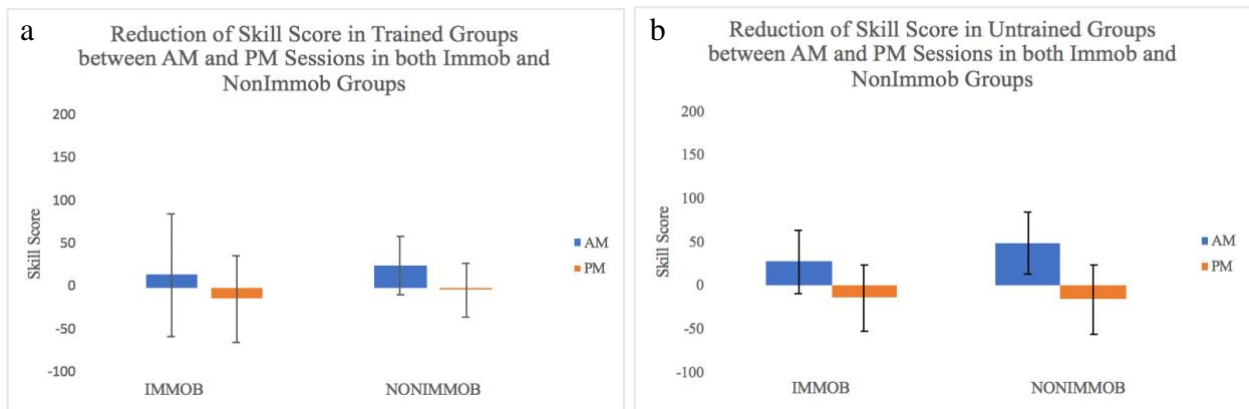
**Statistical Analyses**

Independent two-sample t-tests were performed to determine an immobilization effect on Trained and Untrained groups’ skill scores and change scores.

**RESULTS**

**Skill Score Analysis**

Skill Score (SS) in the right, untrained hand was compared between groups below. Figure 6 summarizes results based on average AM and PM sequence specific task performance. Positive



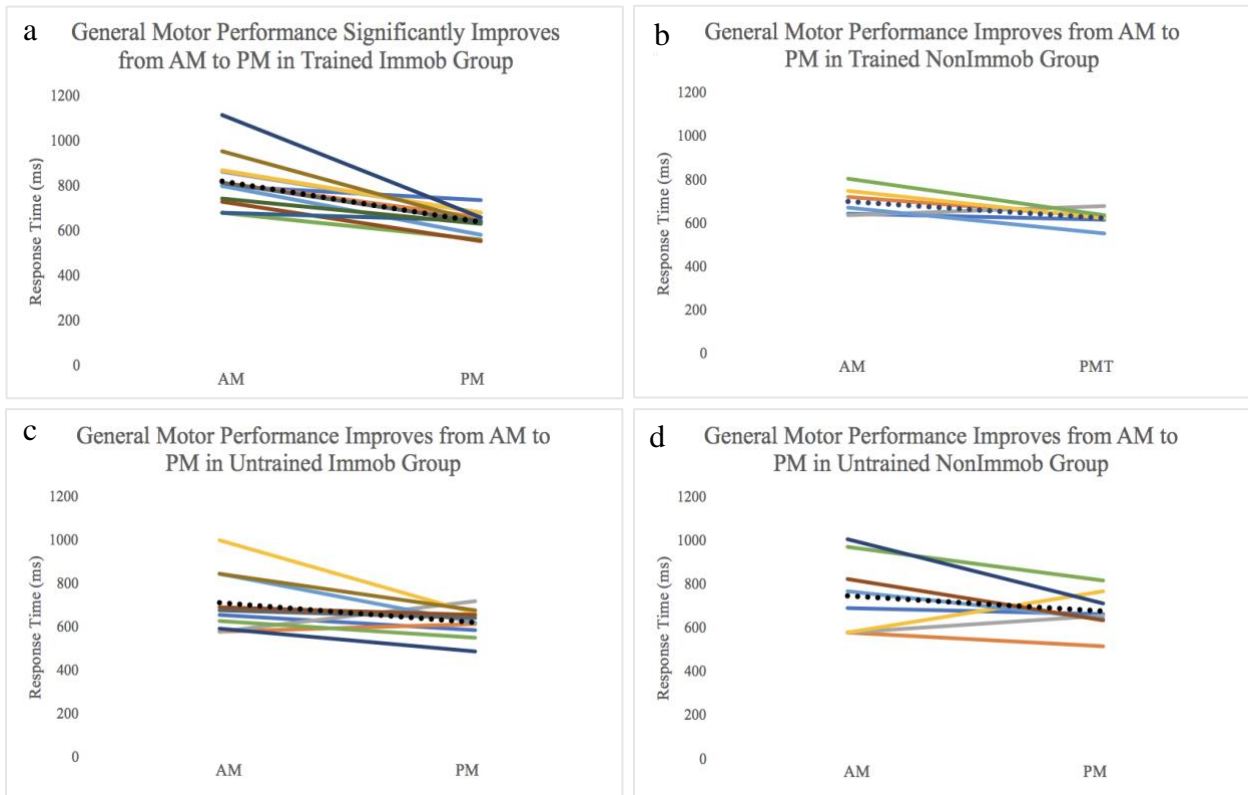
**Figure 6. Skill Score Summary Between Groups.** On average, Skill Score is positive in all AM sessions, and decreased and/or became negative in the PM session in the both the (a) trained and (b) untrained groups.

SS are seen in all groups in the morning session, while negative SS are seen across all groups in the afternoon testing session. There was no statistically significant difference between

immobilization states ( $p=0.8437$ ) nor was there a suggestion that left-hand training significantly increased sequence specific performance ( $p=0.497$ ).

### General Motor Performance

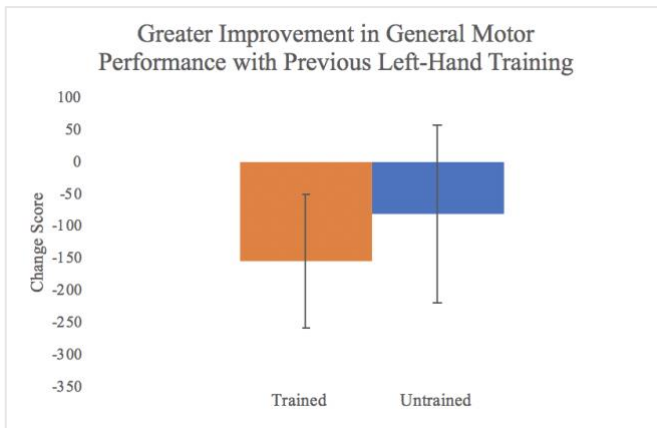
As seen in figure 7, performance during the random button presses in the SRTT improved between skill assessments, as depicted by the decrease in average response time from AM to PM. Immobilization enhanced motor performance in the Trained group ( $p=0.00026$ ) but had no significant enhancement in the Untrained group ( $p=1.005$ ) (figures 7a & 7c). The effect of prior left-hand training was compared between the Immobilized group and Non-Immobilized Group. Left hand training significantly decreased response time and overall general performance in the Immobilized group when compared to the Non-Immobilized group ( $p=0.0419$ ) (figures 7a & 7b). For the Untrained participants, there was no significant difference between the immobilization states ( $p=.8247$ ) (figures 7c & 7d).



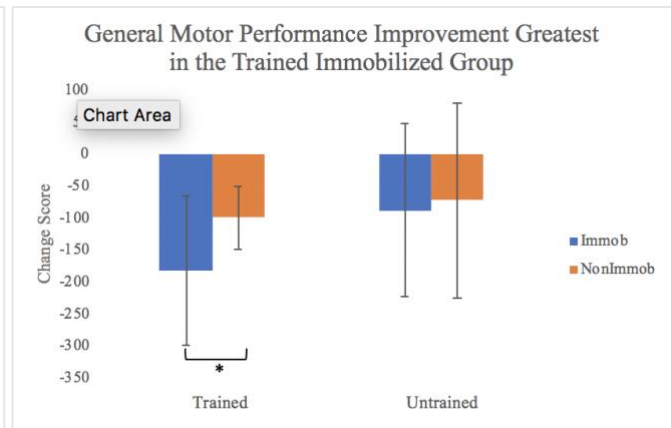
**Figure 7. General Motor Performance from AM to PM.** Response time in the random button presses was higher in the AM and lower in the PM. General motor performance improves from AM to PM in all groups. **4a.** Greatest improvement was seen in the Trained and Immobilized group ( $p=0.0003$ ).

### Change Score Analysis

Change Score (CS) illustrate performance changes between skill assessments in two distinct motor performance areas: the random button presses and the sequenced button presses. Larger reductions in CS suggest reduced response times in the PM and overall improved motor performance between skill assessments. Change in general motor performance was further analyzed in Figure 8 and 9 to illustrate improvement between sessions amongst groups. Figure 8 compares the CS in random SRTT performance between the Trained and Untrained groups. Not taking in account for immobilization state, the Trained groups' random performance CS approached significance ( $p=0.0807$ ). Within the Trained group, random performance between the Immobilized and Non-Immobilized group was compared. The Immobilized group had a significant decrease in change score ( $p=0.045$ ), indicating general motor skill improvements in the task, as shown in figure 9.

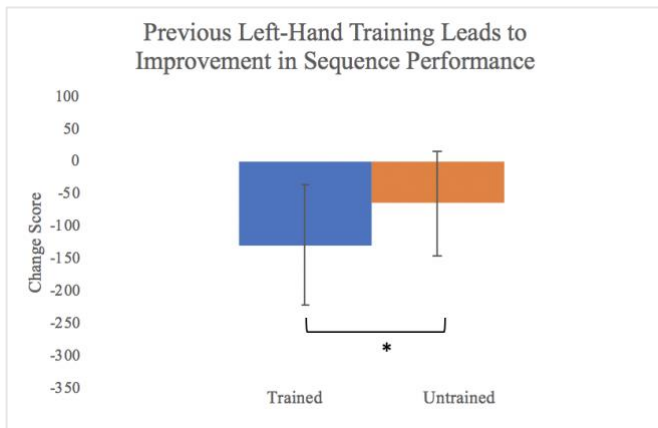


**Figure 8. General Motor Performance Enhanced in Trained Group.** Prior left-hand training enhanced general motor performance in the right hand and approaches significance ( $p=0.0807$ ).

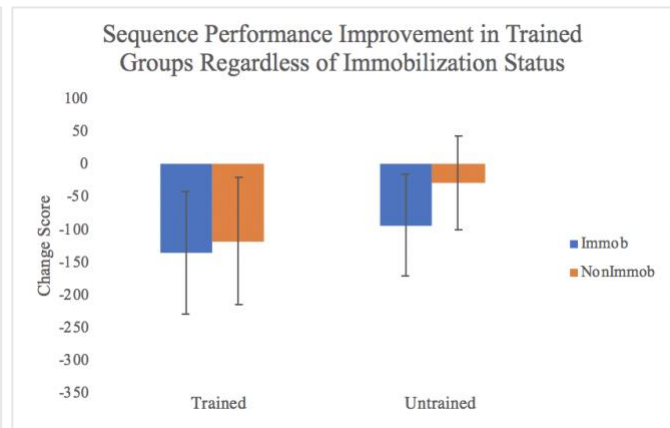


**Figure 9. General Motor Performance Change Score (CS) Across Groups.** CS was greatest in the immobilized trained group ( $p=0.045$ ).

Figure 10 and 11 depict the change score for repeated sequence performance. Response time in the sequenced button presses significantly decreased from AM to PM in the Trained group ( $p = 0.0323$ ), shown in figure 10. There was no significant difference found between the Immobilized and Non-Immobilized Trained groups' ( $p = 0.717$ ) sequence performance (figure 11).



**Figure 10. Sequence Performance Enhanced with Previous Training.** Previous left-hand training enhanced performance in the right hand from AM to PM (0.0323), regardless of immobilization assignment.



**Figure 11. Sequence Performance AM to PM Change Score.** No significant immobilization effect on sequence improvement.

### ***Additional Statistical Analysis***

A two-sample t-test was run to determine a statistical significance between baseline (AM) sequence-specific performance in the Trained and Untrained group. AM SS between the two groups were compared, and no statistical difference was determined ( $p = 0.261$ ). A one-sample t-test was performed to compare sequence-specific AM performance in all groups against the baseline of zero. The t-test determined a significant difference between AM SS and the baseline ( $p = 0.0022$ ).

## **DISCUSSION**

The primary objective of this study was to evaluate the effects of immobilization on the transfer of sequence-specific motor skill training to the non-trained limb. Results suggest there was no significant effect of immobilization on sequence-specific skill transfer but there was significant transfer of general skill from the trained to untrained limb with post-training immobilization. Results suggest that immobilization facilitated general motor skill transfer from left to right hand but was not specific to the sequenced button presses. It is suggestive that 1) left hand task training enhanced right hand sequence performance and 2) immobilization facilitated the transfer of general motor skill in those previously trained.

The results indicate that the transfer of implicit motor skill was not sequence specific. While it is interesting that extensive training on the left-hand improved sequence performance in

the right hand (figure 10), performance on the random button presses also improved. Therefore, it is likely that previously trained participants transferred motor skill to the right hand, not the knowledge of an implicit sequence. The results do not suggest that the consolidation of implicit sequence skill in the left hand was transferred to the right hand, nor that it was facilitated by immobilization. This lack of sequence specific consolidation agrees with the findings of Meier & Cock (2014) and may require an analysis of neurophysiologic variables to understand the lack of effect. Left-hand immobilization only had a facilitatory effect on general motor performance in those previously trained. Why there was no general improvement in the sequenced button presses is unclear. Unexpected results could be due to a number of reasons: the training paradigm, task difficulty, immobilization duration, and/or a time-of-day effect.

The 12-button-sequence pattern (“rep block”) in the SRTT can be configured two different ways<sup>37</sup>, relating to the flashed stimuli on the screen and the button press they correspond to: 1) the finger-button coordination is mapped mirroring the two hands or 2) the finger-button coordination is mapped according to identical visuo-spatial mapping. With “mirroring”, a different button pattern for each hand is presented on the screen in order to activate the same finger movement on each hand; for example, in order to activate the index finger, the left hand is prompted with the stimuli corresponding to the right-most button press, while the right hand is cued to press the leftmost button. Alternatively, a pattern that follows a visuo-spatial mapping pattern is presented with the same paradigm on the screen and cues activation of different fingers—the left-most square on the screen corresponds to the pinky finger on the left hand and the index finger on right hand. A study by Panzer et al. suggests an important distinction between the two patterns of sequence-motor presentation. Same motor-coordinate (“mirroring”) sequence presentation enhanced performance during simple tasks, while a more visuo-spatial coordination was preferred in complex tasks<sup>37</sup>. In the present study, a “mirroring” condition was used, a decision based on findings from Cohen et al. that observed significant off-line consolidation following a mirrored motor sequence learning. It is possible that our task design was more difficult than the design used by Cohen et al., and this could contribute to the difference in findings between the two studies.

Studies have found that a short-term immobilization effect was significant in immobilization periods longer than six hours. Across literature, immobilization periods of 8-10 hours<sup>38</sup> is suggested to be sufficient to modify corticospinal excitability, and other studies

suggest a “short-term” immobilization period of 12 hours<sup>20</sup> or even four days<sup>28</sup>. I speculate that while the six-hour period may have been sufficient to modify corticospinal excitability and enhance the transfer of general motor skill, it may have not been sufficient to transfer sequence skill to the untrained limb.

Across all groups, sequence-specific skill performance in the untrained hand was worse in the evening than the morning (figure 6). This observation contrasts the increase in general motor speed evident in figure 7. The statistical significance of AM baseline skill score ( $p=0.0022$ ) suggests that the 280-button-sequence test block may have prompted acquisition of sequence specific skill. Such a conclusion is questionable as there was only one task exposure, and further analysis is necessary. One reason sequence-specific skill performance was better in the morning could be due to it following sleep more directly than the afternoon session. Enhanced motor performance has been linked to sleep<sup>39</sup>. In addition, a study by Kvint et al. suggested that AM-trained individuals have enhanced rates of skill acquisition compared to individuals trained in a motor-sequence in the afternoon<sup>40</sup>. However, this result by Kvint et al. was exhibited in the number of anticipatory movements in the motor task, which represented declarative (a form of explicit) learning. In the Kvint et al. study’s representation of implicit learning, there did not seem to be a difference between AM and PM trained groups. My present study does not consider the task performance of the trained hand, but only of untrained, right-hand performance. The results of the study by Kvint et al. observe no change in implicit learning sensitivity between sessions on the trained hand. Although Kvint et al. did not study the untrained hand’s performance, my study’s lack of implicit sequence-specific skill improvement relates to Kvint et al.’s findings.

Data collection is ongoing. Motor data of the trained, left hand will be analyzed and compared to right hand general motor performance and sequence-specific skill performance. Activity monitor data collected during the 6-hour immobilization period can be analyzed to see participants’ level of compliance to the immobilization protocol. It would be interesting to see if more compliant participants with uninterrupted immobilization resulted in more robust neurophysiological changes—and, in turn, lead to enhanced skill consolidation—than participants with temporary or more long-lasting interruptions in their immobilization. Moreover, it would be noteworthy to study the neurophysiological changes before and after the immobilization period and correlate it to motor performance results. Additionally, it may be of

interest to study the effect of offline consolidation during a longer period of time after the sessions, such as analyzing sequence performance a day following training. Lastly, it would be interesting to modify the data collection procedure to test for right hand performance after left hand training in the afternoon (the Untrained group). Perhaps short-term immobilization has a facilitatory effect on online task acquisition.

## CONCLUSION

Our results suggest that immobilization enhanced motor performance of the untrained limb after training on the opposite hand six hours prior. However, the current data suggests immobilization does not facilitate inter-limb transfer of implicit *sequence-specific* skill. Those not immobilized after training did not undergo a significant improvement in motor performance, indicating that increased inter-limb transfer of general motor skill is related to immobilization. Our results support findings from early studies<sup>33,34</sup> indicating that implicit learning in the SRTT is limited to motor-based learning, not sequence-specific learning. An analytical comparison between the trained left hand and the untrained right hand in both their general motor and sequence-specific task performance is a necessary next step to evaluate the greater immobilization effect on skill acquisition and consolidation. Further study will investigate the link between immobilization and neural plasticity and may ultimately lead to an enhanced understanding of motor learning augmentation in the impaired brain.

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