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The Effect of Peripheral Nerve Stimulation on Implicit Motor
Learning

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Declaration

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Gabriel Byczynski

Summary

A steady growth of motor learning and electrical stimulation research has produced important advancements, however constrained by methodological and theoretical uncertainty. As the field of motor learning modulation continues to progress, alternative methods and protocols are necessary in order to further our understanding of how stimulation can alter motor learning, and by which mechanism it acts. At the present moment, the exact mechanisms of transcranial alternating current stimulation (tACS) are unknown, and recent studies have indicated that the present hypotheses are physiologically and mechanically undermined. As an answer to this uncertainty, the peripheral nerve hypothesis offers an alternative explanation, or indeed alternative approach, to modulating motor learning using electrical stimulation. Extensive study in rodents have established the feasibility of motor learning manipulation using nerve stimulation and supports its translation to humans. This thesis then presents the first evidence that motor learning can be modulated in humans using stimulation of the occipital nerve. This work compiles a literature review and theoretical proposal of how transcranial mechanisms are explained in the periphery, a meta-analysis investigating regional activation during motor learning tasks, and three experiments which sequentially establish evidence of the peripheral effect of nerve stimulation on motor learning and its subsequent neural and physiological correlates.

Chapter 2 first establishes the theoretical translation of historical tACS research from a transcranial to and transcutaneous mechanism. We discuss the stage-specific effects of stimulation on motor learning, and review the current proposed mechanisms and explanations for modulated behaviour and neural activity. We then proceed to propose how the current hypotheses are constrained by recent methodological studies which sought to analyze the validity of the transcranial mechanism(s). This chapter concludes by putting forth that modulating motor learning using peripheral nerve stimulation is methodologically valid, bolstered by successful pre-translational work.

Chapter 3 describes a meta-analysis which sought to identify online (during) and offline (after) motor learning-related activation in the brain using fMRI. 38 studies were included (32 online, 6 offline) following review, and an activation likelihood estimate (ALE) meta-analysis was performed in order to identify consistently activated regions across motor learning studies. We identified numerous brain regions which were either unique and overlapping during online and offline motor learning tasks. Online motor learning analysis revealed validating activation of regions associated with the adaptive motor learning theory. We consider the results of this analysis as directive in nature for establishing regional targets in motor learning modulation.

Chapter 4 describes the first study in which we paired stimulation of the occipital nerve with motor learning in several frequency conditions to identify if behavioural modulation was

produced, and if so, if it was optimized at a specific frequency. 60 participants (n=15/condition) were initially recruited to determine the effect of sham, 1 Hz, 20 Hz, and 40 Hz stimulation on motor learning during a serial reaction time task (SRTT). 20 Hz stimulation produced significantly greater motor learning as measured by normalized motor skill. An additional 15 participants were then recruited to participate in the study with the addition of a topical anesthetic. We found that lidocaine applied peripherally significantly reduced the effect of 20 Hz stimulation on skill improvement, supporting the involvement of peripheral, but not transcranial, mechanisms.

Chapter 5 describes the first follow-up study, which compared 20 Hz and sham stimulation during a motor task with concurrent electroencephalography (EEG) and pupillometry. 30 participants (n=15/condition) completed the study. We observed significant differences in inter-manual transfer supporting heightened learning in the 20 Hz stimulation condition. Decreased resting state neural connectivity, reductions in event-related potentials, and altered individual peak beta frequencies supported 20 Hz-driven modulation of EEG measures, correlated with behavioural improvement. Pupil constriction was also more prevalent in the 20 Hz compared to sham stimulation group, suggesting some modulation of sympathetic nervous system activation potentially through altered noradrenaline activity. We therefore concluded that 20 Hz stimulation alters behavioural, EEG-correlated, and arousal-based measures of motor learning.

Chapter 6 described the final experimental, which was a preliminary investigation into the effect of 20 Hz stimulation on motor cortex excitability. 10 participants (n=5/group) completed the study in either a 20 Hz active or 1 Hz control groups. Significant differences between pre- and post-stimulation motor evoked potentials (MEPs) by single pulse transcranial magnetic stimulation (TMS) were observed, whereby increased amplitude in the contralateral hemisphere was prevalent in the 20 Hz but not 1 Hz stimulation group.

In summary, this thesis aims to determine how non-invasive stimulation of the greater occipital nerve (NITESGON) might modulate motor learning through peripheral mechanisms, consistent with the adaptive motor learning framework and LC-driven neural gain hypotheses. This work represents the first experimental evidence of modulated motor learning through the occipital nerve and adds to the growing number of investigations into the peripheral effect of brain stimulation. We believe this research has the potential to contribute to the fields of brain stimulation, motor learning, and eventually, clinical treatment.

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Ethics Declaration

The review paper in **Chapter 2** required no ethical review.

The meta-analysis in **Chapter 3** was conducted on publicly available data, and due credit is given where necessary. No ethical review was required.

The empirical studies in **Chapters 4 and 5** were approved by the school of Psychology Research Ethics Committee, Trinity College Dublin, the University of Dublin. All behavioural data (pseudonymized with identifiable information removed) are available online. Written informed consent was collected before the start of the first session. All work was carried out in accordance with the declaration of Helsinki and EU GDPR. In **Chapter 4**, no monetary compensation was provided, however course credit was provided for students as per a recommended research participation scheme. In **Chapter 5**, participants could choose between course credit, or monetary compensation for travel.

The empirical study in **Chapter 6** was performed at the BRAI³N Neuromodulation clinic in Ghent, Belgium. Participants provided written informed consent prior to voluntary participation. All work was carried out in accordance with the declaration of Helsinki and in accordance with EU GDPR.

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List of abbreviations

Ach – Acetylcholine

DA – Dopamine

EEG - Electroencephalography

ERDS – Event-related desynchronization

ERP – Event related potential

GABA – Gamma-aminobutyric acid

Glu – Glutamate

LC – Locus coeruleus

LC-NA/LC-NE – Locus coeruleus – noradrenaline/norepinephrine

MEG – Magnetoencephalography

MRI – Magnetic resonance imaging

NA/NE – Norepinephrine/noradrenaline

NB – Nucleus basalis

NITESGON – Non-invasive stimulation of the greater occipital nerve

ONS – Occipital nerve stimulation

PMBR – post-movement beta rebound

SRTT – Serial reaction time task

TACS – Transcranial alternating current stimulation

TDCS – Transcranial direct current stimulation

TES – Transcranial electrical stimulation

TMS – Transcranial magnetic stimulation

TNS – Trigeminal nerve stimulation

VNS – Vagus nerve stimulation

5-HT – 5-hydroxytryptamine (Serotonin)

Chapter 1 Introduction

1.1 Motor learning in the brain

We are what we repeatedly do, therefore, excellence is not an act, but a habit.

- Will Durant

Humans are born to move; however our existence dictates we must *learn* to do so with grace and skill. Motor learning, in its broad definition, therefore describes the numerous overlapping and adaptive processes which contribute to effective movement and skill (Krakauer et al., 2019). It is no surprise then, that motor learning manifests in its most tangible state (a generous title) in the brain. Numerous regions of the brain are engaged when learning a movement, a task, a behaviour; however the cerebellum, basal ganglia (BG) and primary motor cortex (M1) are arguably the most discussed in motor learning literature. Across numerous species, and all vertebrate brains, the cerebellum (or cerebellar-resembling structure) is generally believed to compute estimates of what will happen after a command is given (Shmuelof & Krakauer, 2011). Thus, the cerebellum allows for *control via prediction*, and in turn can act to adapt to errors and external influence, which clarifies the role of cerebellum in (fast) adaptation in motor learning (Galea et al., 2010; Tzvi et al., 2022). The complete role of the BG are slightly less clear, especially given that causality-supporting non-invasive stimulation studies such as those in the cerebellum (Giustiniani et al., 2019a; Giustiniani et al., 2021) are less practical in subcortical regions. The anatomical BG consist of the striatum, pallidum, subthalamic nucleus, and substantia nigra (Groenewegen, 2003). These regions are subdivided further, however here we need only discuss a more conceptual contribution rather than one with anatomical specificity. The BG and cerebellum function together to instigate movement, adjusted through looping neuronal circuits, consistent with predictive and forward modelling theories (Porcill & Dean, 2007; Thach, 1998). In a logical step, it has then been demonstrated that the motor cortex and other higher-order regions then retain the learned cerebellar skill, capturing in essence the transitive process by which motor skill is acquired, learned, and retained (Galea et al., 2010).

Knowing which regions contribute to motor learning, and particularly how these regions function to produce the learning and later retrieval, is important particularly when aiming to develop treatments and interventions when motor learning fails or when a set-back occurs. This thesis intends to focus on this point, namely the development of an intervention which modulates motor learning for future clinical, practical, and interventive utility.

1.2 Modulating motor learning

Given the importance of motor ability in quality of life, both through periods of development and in recovery following illness, there is a considerable breadth of literature which has sought to alter the strategy and extent to which we as humans learn a movement. Many methods exist both theoretically and experimentally, including TMS (Richards et al., 2008), tES (Meng et al., 2024), general rehabilitation, the engagement of other cognitive strategies (Dobkin, 2004), and inter-modal transfer (Ghai et al., 2019), to name a few. There is a natural variability within all of these approaches, but the end goal seems to settle on the overall improvement of some behaviour, which will improve to some extent the quality or ability of the patient. This thesis will primarily focus upon electrical stimulation; but it is noted that the future of motor modulation in rehabilitation is more than likely a personalized combination of any number of techniques (Cha & Hwang, 2022; Sharififar et al., 2018).

The proposed mechanistic methods by which tES is believed to act, and how this modulates brain function, are further described in detail within **Chapter 2**. As a brief introduction, tES is broadly the use of electrical current on the scalp to alter brain activity (Reed & Cohen Kadosh, 2018). Using direct current stimulation, one can alter (often increase) resting membrane potentials in targeted regions, increasing the probability of neuronal firing, which in turn can alter plasticity and activity within the necessary brain regions under target. Alternating current stimulation (tACS) instead uses a sinusoidal alternating current waveform which is believed to entrain or rhythmically alter neural oscillations, similarly for the purpose of altering endogenous activity such as connectivity (Antal & Paulus, 2013). Given the importance of regions such as the motor cortex (specifically in cortical plasticity) for motor learning (Di Lazzaro

et al., 2009; Li Voti et al., 2011; Ramanathan et al., 2006), it is then no surprise that many of the current experimental approaches to modulating motor learning consider targeting the motor cortex with brain stimulation. The overall goal therein is to alter cortical plasticity with direct current stimulation, or modulate motor network connectivity via alternating current stimulation, with the eventual application of improving pathological motor dysfunction, or boost inherent motor ability. Despite this extensive history, the exact mechanism by which tES acts, and its success in modulating motor learning, are continually put into question (Bland, 2021; Burkhardt et al., 2024; Vöröslakos et al., 2018), priming other research fields to investigate in more basic neuroscientific paradigms, the alternative hypotheses (Asamoah et al., 2019; Byczynski & Vanneste, 2023) and methods (Luckey, McLeod, Huang, et al., 2022; van Boekholdt et al., 2021). One alternative approach is the peripheral nerve hypothesis (Asamoah et al., 2019). In this theory, the contradicting aspects of 1) successful modulation of brain activity and 2) methodological investigations suggesting electrical current struggles to reach the brain at classic intensities (Ibáñez et al., 2023; Vöröslakos et al., 2018), are synthesized such that an alternative route for the current through the peripheral nerves of the head is proposed. This route would posit that stimulation of peripheral nerves increases activity at the afferent connection to the brainstem, through the Locus coeruleus (LC), and onward via the LC-NE pathway in the brain (Figure 1.1).

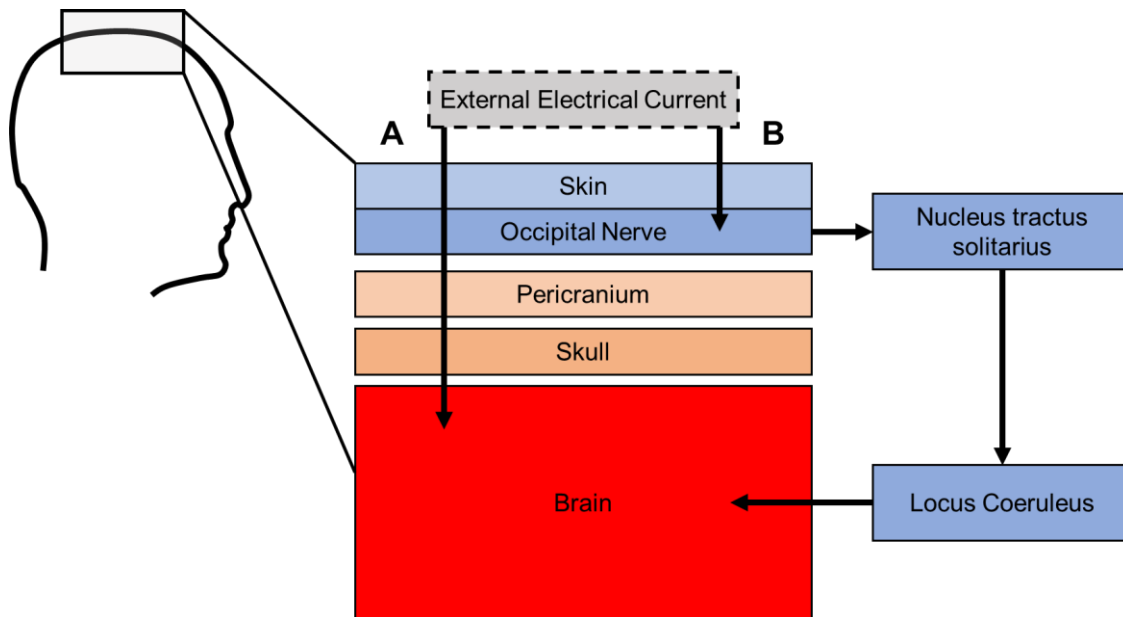


Figure 1.1 Proposed peripheral pathway for tES. (A) Transcranially and (B) transcutaneously.

1.3 Translating rodent models

In fundamental neuroscience, novel motor modulation research has been conducted using an adjacent but distinct form of electrical stimulation in VNS. Many studies have been carried out investigating VNS for motor improvement specifically (often following stroke) (Buell et al., 2018; Hays et al., 2016; Hays et al., 2015; Daniel R. Hulsey et al., 2016; Hulsey et al., 2019; Khodaparast et al., 2013; Khodaparast et al., 2016; Meyers et al., 2018; Pruitt et al., 2021). Throughout this research, it has been established that in rodents, pairing electrical stimulation of the vagus nerve with a motor task improves motor recovery greater than rehabilitation or VNS alone, and enhances cortical map plasticity (Khodaparast et al., 2013; Khodaparast et al., 2016; Meyers et al., 2018). In searching for the mechanism, it was discovered that the effect of VNS on motor map plasticity is attributable to multiple neurotransmitters including serotonin (5-HT), dopamine (DA), acetylcholine (ACh), and norepinephrine (NE) (Brougher et al., 2021; Daniel R. Hulsey et al., 2016; Hulsey et al., 2019; Tseng et al., 2021). Accordingly, the LC and more generally the LC-NE system became of interest, given that the LC represents the main producer

of NE, and is a key mediator of the central nervous system (Hulsey et al., 2017). This point is particularly salient given the role that the LC-NE system plays in performance, engagement, prediction error, and general neuroplasticity (Jordan, 2024; Rommelfanger et al., 2007). Martin et al. (2024) also showed that VNS improved perceptual learning via activation of the cholinergic neural inputs to the cortex, consistent with cholinergic involvement in motor plasticity. Indeed this then becomes the basis for the following work, as it establishes that peripheral (vagus) nerve stimulation can modulate not only motor map plasticity, but also possesses a widespread control across other domains which have been established as important for motor learning specifically, such as the adaptive motor learning framework (Aston-Jones & Cohen, 2005a, 2005b; Porrill & Dean, 2007; Shadmehr, 2018; Tani et al., 2014).

1.4 Translating research in peripheral nerve stimulation

In parallel to the rodent-based work establishing the vagus nerve as a suitable target for motor plasticity modulation and stroke recovery, peripheral nerve stimulation has also grown from previous clinical roots into modern experimental paradigms. Occipital nerve stimulation, a form of neuromodulation using electrical stimulation which was originally developed to treat migraine, is one example of how peripheral nerves are now being targeted for neuromodulation (Montenegro & Kisson, 2023). As a step into investigating its peripheral effect, the first study using non-invasive stimulation of the greater occipital nerve (NITESGON) was carried out in 2020 (Vanneste et al., 2020). Stimulating the occipital nerve improved memory in humans, which supported the involvement of the LC-NE system and validated nerve-specific effects. As an establishing experimental work, this study illustrated that it was possible to modulate cognition using stimulation of a the occipital nerve, and fitting with previous research, that this effect followed the proposed LC-NE pathway similarly described in rodents (Hulsey et al., 2019). Since then, other studies have successfully investigated the effect of NITESGON on memory in older adults, and with varying stimulation parameters (Luckey, McLeod, Huang, et al., 2022; Luckey, McLeod, Mohan, & Vanneste, 2022). Accordingly, there are then two origins for pursuing the translation of the motor studies in rodents into humans: firstly, the ability to activate the LC-NE

system via the occipital nerve, and secondly, the known effect of peripheral nerve stimulation on motor ability and recovery via the LC-NE system in rodent models. This thesis therefore intends to pursue the synthesis of these ideas to translate rodent motor discoveries into human research. The compiled evidence thus far, and theoretical explanation I intend to present, summate into the following theory: NITESGON delivered during a motor task modulates LC activity resulting in changes in the LC-NE pathway driving behavioural motor changes. These changes, according to the adaptative motor learning framework, would modulate neural gain and alter the balance in sensory and predictive input, driving enhanced neuroplasticity as has been observed previously (Mather et al., 2016). As a result, motor learning behaviour would be modulated in individuals receiving NITESGON.

1.5 Hypotheses and aims

This dissertation aims to investigate the specific effect of peripheral nerve stimulation on motor task learning. Here, I take a structured approach to determine its effect by building on theoretical framework to develop experimental approaches which subsequently identify the behavioural and neural effects of peripheral nerve stimulation on motor task learning behaviour and its neural correlates. Here, I propose the following nesting hypotheses and the chapter in which the hypothesis is addressed:

- i) Theoretical transcranial effects can be explained via peripheral mechanisms
(Chapter 2)
- ii) Online targeting is suitable for modulating motor circuitry based on meta-analytical fMRI correlates (Chapter 3)
- iii) Occipital nerve stimulation modulates online motor learning behaviour
(Chapter 4)
- iv) Motor learning behavioural changes result from modulation of neural activity
(Chapter 5)
- v) Plasticity changes fundamentally drive the modulation of neural activity and behaviour (Chapter 6)

1.6 Theoretical and experimental approaches

This dissertation compiles several works including a review/opinion paper, meta-analysis, and three empirical chapters which cooperatively aim to investigate the effect of peripheral nerve stimulation on motor task learning.

In **Chapter 2**, I begin by reviewing the current theory and history of modulating motor learning with transcranial alternating and direct current stimulation, in order to identify the current theories of how stimulation may alter motor learning, and identify parameters which appear to moderate the effects shown, such as stimulation timing and task-pairing. I discuss how the current theoretical bases of transcranial stimulation can be translated to support a peripheral or transcutaneous mechanism, which I then leverage to direct my subsequent experimental investigation.

In **Chapter 3**, in order to further establish regional and temporal stimulation targets (Takeuchi & Izumi, 2021) and elucidate the stage-specific processes of motor learning (Dahms et al., 2020; Weaver, 2015), myself and two independent reviewers perform an activation likelihood estimation (ALE) meta analysis of motor learning during online (during task) and offline (after task) scanning. A coordinate-based meta-analytical approach was used following review and identification of n=517 studies, of which n=36 were included in the final analysis. I describe activation similarities and differences between neural correlates of online and offline motor learning, and discuss the role of the findings in directing stimulation theories and experimental design. I also describe robustness checks and the extent of which we replicated previous findings. Taken together, **Chapter 2** and **Chapter 3** cooperatively identify theoretical targets and parameters used to direct experimental investigation.

In **Chapter 4**, I described the first of three experiments, designed to initially determine if occipital nerve stimulation modulates motor learning behaviour. We recruited participants (n=60) randomly assigned to sham, 1 Hz, 20 Hz, or 40 Hz occipital nerve stimulation groups. Participants performed a 15 minute serial reaction time task (SRTT) while concurrently receiving stimulation, and returned 24 hours later to perform a re-test of the task on both trained and

untrained hands. Here, I describe the motor learning task, stimulation methodology, and behavioural changes which were produced as a result of stimulation of the greater occipital nerve. I also discuss the role of strategy and the theoretical implication of frequency-specific effects for motor learning modulation. In a follow-up to the first experiment, I then recruited an additional 15 participants to complete the task with concurrent NITESGON at the optimal frequency, while also being pre-treated with a topical anaesthetic. Here I describe the effect of nerve blocking on the mechanism of NITESGON. These findings are then used to design the subsequent studies described in the next chapters.

In **Chapter 5**, I describe the second of three experiments, which built on the findings of **Chapter 4**. We recruited participants ($n = 30$) randomly assigned to either sham or 20 Hz occipital stimulation groups. Participants performed a 30-minute extended version of the original 15 minutes SRTT task, with concurrent stimulation and electroencephalography (EEG). Resting state EEG was recorded before and after the task on day 1. EEG was recorded during the entirety of the task on day 1 and day 2, while stimulation was delivered during blocks 2-4 during day 1 only. I describe modulated motor learning behaviour differences between the sham and active groups. I also discuss changes in resting-state EEG connectivity, event-related potentials (ERPs), and correlated behavioural and neural measures. I conclude by discussing how neural correlates and behavioural changes together suggest a distinct mechanism by which stimulation alters motor learning.

In **Chapter 6**, I describe the final of three experiments, which built on the findings of **Chapter 4** and **Chapter 5**. In an identical task protocol to **Chapter 5**, we recruited participants ($n = 10$) randomly assigned to receive 20 Hz or 1 Hz occipital nerve stimulation. We recorded motor-evoked potentials before and after training and testing. Here I describe modulated motor evoked potentials following stimulation, which were not recovered in the 1 Hz control group. I also discuss the effect of age and population heterogeneity on behavioural correlates of motor learning and its impact on stimulation parameters and utility.

In **Chapter 7**, I conclude the thesis by summarizing the thesis and addressing the original hypotheses. I discuss the implication of these findings for motor learning research, and its application to motor recovery and treatment for stroke and other movement-related dysfunctions. I specifically discuss the potential utility NITESGON may hold for future motor rehabilitation and training, in the context of electrophysiological changes and altered metrics of plasticity in both behavioural and physiological manifestation.

Chapter 2 Modulating motor learning with brain stimulation: Stage-specific perspectives for transcranial and transcutaneous delivery¹

2.1 Introduction

In order to first address the numerous theoretical and practical variations in modulating motor learning with brain stimulation, this chapter reviews the use of transcranial alternating and direct current stimulation on motor learning. With the growing popularity of non-invasive electrical brain stimulation techniques like transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS), researchers have progressed significantly in discovering applications, proposing mechanisms, and producing interventional therapies. Non-invasive electrical stimulation of the brain is a method that employs the use of a mild electrical current between electrodes while in contact with the scalp. It has been used for the treatment of psychiatric disease (Buchanan et al., 2020; M.-F. Kuo et al., 2017), improving memory (Jo et al., 2009; Luckey, McLeod, Mohan, & Vanneste, 2022), tinnitus (Vanneste & De Ridder, 2011), ADHD (Buchanan et al., 2022), and motor learning (Pixa & Pollok, 2018) with relatively good success. Specific to movement and motor learning, tDCS has also been used in a range of pathological contexts including Parkinson's disease, motor stroke, and multiple sclerosis (Lefaucheur et al., 2017). Thus, brain stimulation has created an entire novel genre of neuro-modulatory research that focuses on determining new avenues which it can be applied. As research progresses, it refines the use and innovation of stimulation methodologies to achieve the most effective

¹ The following chapter incorporates material which has been published in Byczynski, G., & Vanneste, S. (2023). *Modulating motor learning with brain stimulation: Stage-specific perspectives for transcranial and transcutaneous delivery*. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 125, 110766. <https://doi.org/https://doi.org/10.1016/j.pnpbp.2023.110766>.

The author of this thesis is the first author on the publication.

results. Since there are numerous reviews on the general background, mechanisms, and applications of tDCS/tACS, we put forth a focused account of brain stimulation for motor task learning modulation with novel focus on the stage of motor learning in tDCS, tACS, and peripheral stimulation.

In this chapter, I aim to provide an overview of tDCS and tACS and their use in stage-specific motor learning, including a review of the current methodologies and theories in motor learning and the variation between central and peripheral (i.e., transcranial and transcutaneous respectively) delivery during different stages of motor learning. We present a comparison between the alternating and direct current stimulation, and the proposed mechanisms by which the different approaches are considered to act by. We focus our approach by methodologically categorizing results based on the motor learning stage targeted, and the proposed mechanisms of stage-specific modulation. Finally, we perform the same approach for novel peripheral nerve stimulation, drawing parallels between stage-specific effects and mechanistic similarities to transcranial stimulation. Conclusively, we aim to provide an effective account of current, stage-specific, motor task learning modulation with electrical stimulation that supports further, and indeed more focused exploration.

2.2 Motor task learning

Motor learning can be broadly divided into two categories; implicit and explicit learning, often described in terms of the type of underlying motor system or the type of memory involved (e.g., procedural and declarative, respectively) (Kleynen et al., 2014). It is noted however, that it is often the case that explicit motor-learned tasks settle into the implicit, such that paradigms attempting to sequester the implicit aspect of motor learning may still have contributions in the explicit learning sphere (Krakauer et al., 2019). Implicit motor learning is described as the ‘unconscious’ aspect of motor learning,

and is considered to be governed by procedural memory, where the participant cannot consciously recall the learned movement or sequence, however still exhibits training effects (Kal et al., 2018). Conversely, explicit motor learning is considered to be a conscious learning of a motor task, akin to declarative memory (Kal et al., 2018). In this way, the subject is aware of the learned movement or sequence, such that a component of performance relies on consciously recalling the sequence or movement. Motor tasks used in research can be tailored to investigate either implicit, or a combination of explicit and implicit learning. As such, when developing a motor task, researchers tend to form a consensus on how the task should be implemented, instructions given, and how learning is measured, in order to better conclude which aspect of motor learning is being measured (Kleynen et al., 2015).

The distinction between implicit and explicit motor learning is particularly relevant when performing research that aims to understand how neuromodulation can influence motor learning. Implicit motor learning is less ‘at-risk’ of deficits brought on by external cognitive factors. This allows for the use of dual-task paradigms, while preventing factors like fatigue or distraction from interfering with results in implicit motor learning, as is shown to occur during explicit motor learning (Rieth et al., 2010). In a recent review, the contributions of implicit and explicit aspects of motor learning are further discussed (Krakauer et al., 2019). With these considerations, a distinction between implicit and explicit motor learning will be used in this thesis. However, since evidence shows that tasks learned explicitly will have implicit components, we will proceed with the understanding that it is not possible to state that the processes are distinct and non-interacting.

Motor learning is also often divided into semi-structured stages, with characteristics pertaining to the behavioural implication. The first stage, *fast learning*, is the initial skill acquisition of a motor task (e.g., learning the sequence of a 4-button press task), and often occurs rapidly, showing fast improvement in measures such as reaction time and accuracy (Dayan & Cohen, 2011). Fast learning itself is a sub-component of *online learning*, which describes improvements in motor skill during training. Online learning is counterpart to *offline learning*, which reflects skill improvement that occurs after the training, but not during (Figure 2.1). Also, a component of online learning is *slow learning*, which involves a form of ‘refining’ the task that was learned in the fast stage. In this stage, the rapid acquisition of motor skill is reduced to a fine tuning of skill performance, and a general plateau in performance improvement. Interestingly, it is believed that fast learning is generally explicit learning, while the slow learning is implicitly governed (however this is specific to the task) (Dahms et al., 2020).

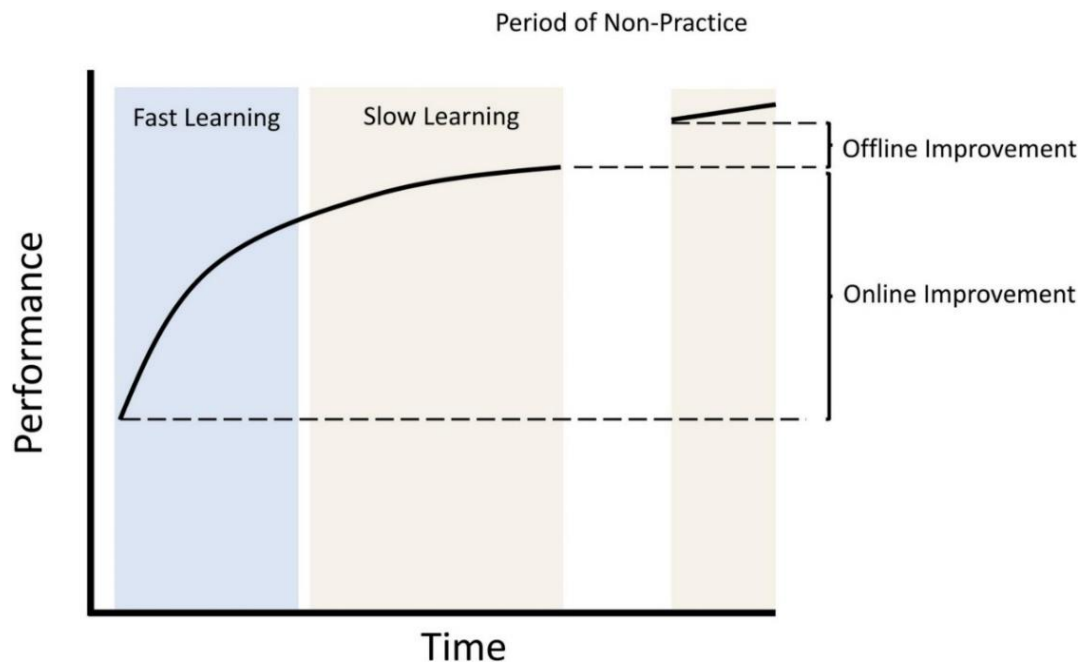


Figure 2.1 Motor learning stages and related performance improvements. A schematic of motor task learning and corresponding fast and slow learning stages. A sharp increase in performance is seen during fast learning, which plateaus during slow learning, reflecting slower performance increases during refinement. A gap in which performance improves without practice reflects offline learning, while any improvement during practice is referred to as online improvement.

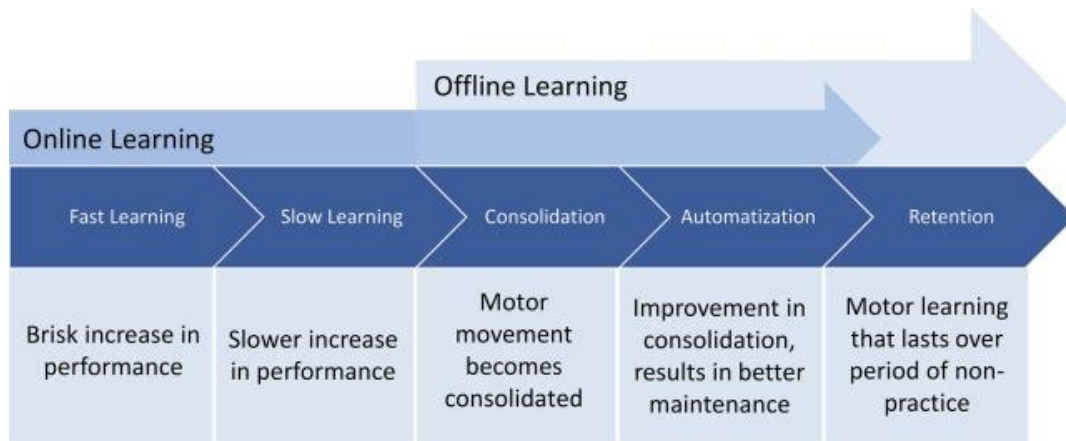


Figure 2.2 Motor learning stages in online and offline learning. The stages of online and offline motor learning which including fast learning, slow learning, consolidation, automatizing, and retention. Despite being shown as individual and distinct stages, there are likely cooperative and overlapping mechanisms which govern their activity.

Consolidation is often considered to follow slow learning. Consolidation is the stage that evokes the offline learning by aptly consolidating the learned motor task (Doyon & Benali, 2005). As a result, performance increases are found to occur after practice, provided no interfering task is introduced. Interestingly, some research has suggested that offline gains are only prevalent in explicit motor learning, and are not found in implicit motor tasks, since offline learning often improves aspects of declarative but not procedural memory in motor skill acquisition (Van den Berg et al., 2019). *Automatization* is a stage in which gains in motor skills are less likely to be influenced by external factors, such as typing in your pin while speaking to the cashier or dialling a friend on the phone while simultaneously having a conversation. There is preliminary evidence that implicit motor task learning leads to better automatization compared to explicit motor learning, although further research is needed to determine the accuracy of this claim (Kal et al., 2018). Lastly, *retention* describes the ability to perform the learned task with minimal losses after a prolonged period of non-practice, akin to an annual bike-ride (Doyon & Benali, 2005). Research investigating retention with stimulation may

therefore attempt to determine how stimulation of the brain increases the length of time and effectiveness of the retention step (Lum et al., 2018).

We propose that by using a stage-based approach, researchers hypothesizing mechanisms and modulation effects can refine their approach to stage-specific targets. It is therefore necessary to consider the function, mechanistic, and behavioural contributions of each learning stage individually, to effectively approach motor learning. This is especially relevant when employing brain stimulation (e.g., tDCS) to modulate learning, as the effect of modulation may differ depending on the stage. Since there are many factors which influence the outcome of the modulation, we will first discuss the possibilities for location of delivery, type of stimulation used, and the stimulus parameters used.

The next sections will discuss individually the use of tDCS, tACS, and peripheral nerve stimulation for motor task modulation, with distinct categorization based on motor learning stage. Alongside results, the sections will also include the influence of stimulus parameters mentioned above such as location of delivery, duration, task chosen, frequency (for tACS), and duration. We will also provide stage-specific mechanistic hypotheses for modulation, with evidence and criticism.

2.3 Electrical stimulation and motor learning

Beginning with location of delivery of the electrical current, a common choice in motor task studies is to stimulate the primary motor cortex (M1) (Giustiniani et al., 2019a; Krause et al., 2016; Reis & Fritsch, 2011). Some other brain regions that have been targeted include the premotor cortex, dorsolateral prefrontal cortex (DLPFC), supplementary motor areas (SMA), superior temporal gyrus, posterior parietal cortex, primary visual cortex, cerebellum, and middle temporal visual area (Buch et al., 2017; Ehsani et al., 2016; Miyaguchi et al., 2022; Zhu et al., 2015). The general motivation in

choosing a region is that researchers suspect it has relevant function in the acquisition, consolidation, or retention of motor task learning. Delivery to M1 is intuitive, due to its essential contributions to motor learning (particularly sequence learning) (Hamano et al., 2021). Stimulation of the DLPFC might be chosen because of evidence suggesting that the DLPFC is involved in acquisition and expression of learned implicit sequences (Vékony et al., 2022). Similarly, the SMA may be targeted because it has been shown that SMA activity increases during motor skill learning (Vollmann et al., 2013). Given the variability of motor learning tasks, types of motor learning (e.g., implicit vs explicit), stage and cortical placement of stimulus delivery, the stimulation settings (i.e., frequency, current, duration), and choice study subjects, there is a wealth of possibility for applications.

TDCS uses a direct and constant current to deliver stimulus to the brain at placed electrodes. The electrode placement, as mentioned previously, largely contributes to the effect of the stimulation as the current travels from one electrode to the other, while arcing through the underlying neural region being targeted (Unal et al., 2020). Due to the nature of electrical current, researchers have the choice between anodal and cathodal stimulation (a-tDCS and c-tDCS respectively). Anodal and cathodal describe which electrode is placed over the region of interest, with anodal stimulation being placement of the positive end, and cathodal the negative. Using this, researchers can choose to either induce either hyperpolarization (at the anode) or depolarization (at the cathode) of the neurons under effect (we further elaborate the mechanism in the forthcoming sections).

A-tDCS and c-tDCS have generally been shown to produce differing results, as for example research has shown that a-tDCS improved motor performance while c-tDCS improved offline learning (Christova et al., 2015). While studies have found motor learning improvements from tDCS, the specific effect of a/c-tDCS on motor learning isn't

necessarily consistent. Some studies report improvement with both a/c-tDCS, (Ciechanski & Kirton, 2017; Khedr et al., 2013), while others report no improvement (Wiltshire & Watkins, 2020). In most cases, positive impacts on motor learning are seen in the anodal stimulation, and not cathodal - corroborated by studies finding motor learning improvements with anodal stimulation (Bastani & Jaberzadeh, 2012), and instead reduced excitability in the M1 cortex from cathodal stimulation (Brunoni et al., 2012; Sasaki et al., 2016). Other research however, have found only partial support, showing anodal stimulation increases motor excitability, and cathodal stimulation resulting in minimal inhibitory effects (Jacobson et al., 2012; Roche et al., 2015). Interestingly, it may be that these contradictory results arise from the differential activation and inhibition effects acting on cortical neurons *or* inhibitory interneurons. In this way, an inhibitory cathodal stimulation that acts on inhibitory interneurons would produce the inverse effect seen when acting on cortical neurons (Lang et al., 2004). Further research would need to quantify the possibility of this claim, and furthermore devise a way to distinguish between the two, so that researchers may be more aware of the neuron type that they are stimulating. Therefore, while considering the historical connotation of a/c-tDCS in this review, we also are conscious that there are inconsistencies in evidence that refute any definite excitatory or inhibitory activity.

It may be that to better understand the complexity of a/ctDCS, better mechanistic understanding is necessary of tDCS in general. One plausible mechanism is that tDCS changes the distribution of charge polarization across the neuron, creating a biased input of incoming connections, and changing the excitability of the cell (Rahman et al., 2013). Nitsche and Paulus also demonstrated results that corroborate a likely effect on the resting membrane potential, resulting in increases or decreases in excitability (Nitsche & Paulus, 2000). Imaging studies have revealed that increases in the blood oxygen level dependent

(BOLD) signals in the M1, DLPFC, and SMA regions are associated with acquisition and retention of motor skill learning (see also review by Dayan and Cohen (2011)) (Lefebvre et al., 2012; Meehan et al., 2011). This hypothesis would therefore also be consistent with the sometimes ‘inverted’ effect of c-tDCS, that is, tDCS elicits its effect on the membrane potential of either cortical or inhibitory neurons, and therefore may produce opposing results. TDCS has also been suggested to improve motor learning by acting with brain-derived neurotrophic factor (BDNF). BDNF is a protein in the neurotrophic family that is established as having a role in development and synaptic plasticity (Bramham & Messaoudi, 2005). BDNF literature in plasticity is lacking however, and so its role in long-term potentiation (LTP) and subsequently motor learning is unclear (Bramham & Messaoudi, 2005). Generally, BDNF secretion is believed to occur throughout motor learning, and research has shown that tDCS improvements in motor learning are BDNF-dependent in mice (Fritsch et al., 2010). While some studies have implicated BDNF in motor learning in humans using a BDNF Val66Met genotype, grouped by homozygosity or heterozygosity (McHughen et al., 2009), other studies have found that it is not necessarily an effective predictor of motor learning alterability (Li Voti et al., 2011).

Due to the lack of understanding the exact mechanism of tDCS, stage-specificity is a relevant avenue to pursue to improve the understanding, and to direct research to specific targets. As such, the stages of motor learning, reduced here to acquisition, consolidation, and retention, may be approached as distinct period which differ in mechanism and thus in their receptivity to modulation. Here, we pursue each stage individually, highlighting the results of current research, proposed mechanisms, and stage-specific methodologies.

The first application of tDCS during motor learning is delivering stimulations during the motor learning task itself, possibly with the intent to improve the rate of

learning, or effectiveness of automatization. We choose to combine fast learning, slow learning, and automatization stages under the general term of acquisition, as these are all aspects of online learning, and are generally suggested to follow similar processes, that is- modifications or refinements that occur during the task. Nitsche and colleagues found that when delivering anodal and cathodal tDCS to the primary motor cortex during a serial reaction time (SRT) task, reaction times decreased faster over trials, and measures of skill (the sharp increase in reaction time when blocks change from sequence to random stimulus) were larger (Nitsche et al., 2003). In another study, a-tDCS delivered over M1 in patients with Parkinson's disease was shown to improve skill acquisition, with improvements retained into the following week (Horiba et al., 2019). Thus, during motor skill acquisition, tDCS research has an agreement on the consensus that performance increases with (anodal) stimulation. Neurochemically, since GABA is often reduced during motor learning, it would be unsurprising that a-tDCS, which is suggested to reduce GABA concentrations, would correlate with improved motor learning (Stagg et al., 2011). In fact, there is evidence that the relationship between GABA and tDCS effectiveness is quite clear; as research has shown that the magnitude of GABA decreases in the M1 region brought on by a-tDCS correlates positively with motor learning and furthermore, fMRI signal changes (Stagg et al., 2011). Therefore, during motor acquisition, GABA decreases are seen resulting from tDCS. It is necessary however, to state that while research has indeed shown GABA decreases resulting from tDCS, that these GABA measures are not necessarily related to excitability. In fact to our knowledge, there have been no direct relationships shown between tDCS-altered GABA and cortical excitability (Bachtiar et al., 2015).

We emphasize that the number of papers that report significant motor task improvements during online learning with stimulation are limited compared to the

number that report after-effects, or effects on testing/consolidation/retention. One study employed stimulation before and during motor task learning, and reported that delivery before the task improved acquisition of implicit learning, while delivery during the task did not elicit improvements (Kuntz et al., 2016). This trend has been shown in other research, as Fujiyama also reported that preconditioning tDCS improves the effect on skill acquisition (Fujiyama et al., 2017). While several studies fail to report significant during-acquisition (online) improvements with stimulation, it is common that follow-up testing is successful in detecting improvements, suggesting alteration to the consolidation of the learned task (Ehsani et al., 2016; Lang et al., 2003; Samaei et al., 2017). We further elaborate this apparent ‘delay’ in tDCS effectiveness in the coming section and visualize the effect in Figure 2.3. Due to the trend of an apparent ‘delayed’ tDCS effect, there are few studies that report significant online learning improvement during tDCS in the initial visit.

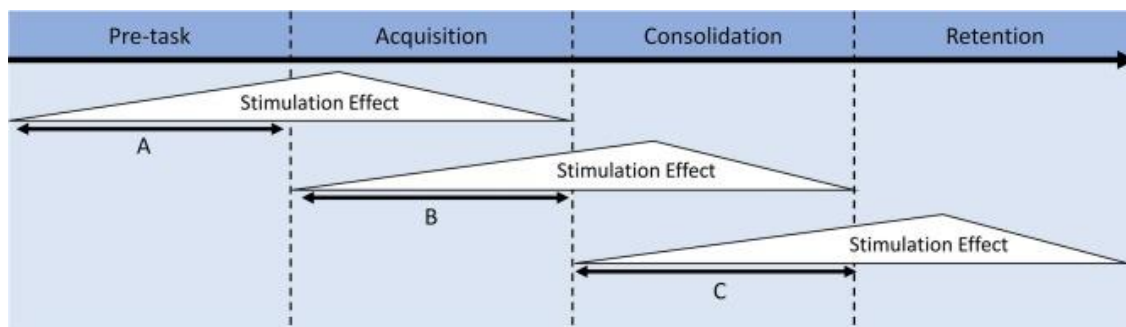


Figure 2.3 Possible interaction between brain stimulation effect and motor learning stages. A schematic showing pre-task, acquisition, consolidation, and retention stages of motor learning. Arrows indicate the duration for which stimulation is delivered, and the triangle represents a theoretical effect strength that results from delivery. This illustrates a theoretical pattern by which the greatest effect of the stimulation may not occur during the stage concurrent with stimulation, and may instead elicit effects after stimulation has ended.

Stimulation during the consolidation period of motor learning is used when attempting to improve the consolidation of the motor task learned, such that during for

example a training/testing paradigm, improvements are seen in the testing occur after the training. Since relatively few studies focus their stimulation during the ‘consolidation’ period alone, it is possible that some of the effects seen on consolidation are due to excitation of the motor region that outlasts the stimulus delivery during acquisition and persists into consolidation. Research using a dual-tDCS (excitation of one hemisphere, and inhibition of the other) found that motor task performance was improved 24 hours after task, but not 1 hour after, providing support for a persisting excitation effect that influences consolidation of the learned motor task when delivered during the task, but not necessarily acquisition (Koyama et al., 2015).

It is interesting that while some studies report improvements during the task itself, and others report improvements in consolidation, in both cases, stimulation is typically delivered during the task, or during the online component. Nitsche and others reported an increase in both task acquisition and early consolidation of the SRT sequence in the tDCS group, attributing this finding to implicit motor learning modulation, and only when delivered to the motor cortex (Nitsche et al., 2003). The significant difference in performance between stimulation and control groups came in later blocks (blocks five and six). These findings suggest that the excitatory effects elicited by tDCS began not immediate, but requires a period of time to ‘ramp up’ and produce modulatory influence. This would explain why some studies report improvements only after the task, suggesting the task durations were shorter than the ‘ramp up’ period, and therefore were not long enough to see the elicited effects while measurement occurred during task. This would also explain why preconditioning with tDCS produces measurable online effects on acquisition, as it may effectively shorten, or shift earlier, the ‘ramp up’ period. A visualization of the possible effect is shown in Figure 2.3, where the onset of the stimulus at the start of the learning stage, does not necessarily indicate that peak of effect will also

occur in that stage. Evidence from tDCS in mice corroborates this story, showing motor cortex excitability increased for a period of time after stimulation was stopped (Fritsch et al., 2010).

When investigating the after-effects of tDCS in humans, as is discussed in a paper by Roche and colleagues, GABA concentration is shown to decrease after anodal stimulation, while glutamate is left unchanged (Roche et al., 2015). Furthermore, there is evidence that GABA synaptic activity is modulated by tDCS and motor learning synergistically, in a paradigm delivering tDCS after motor learning, and not during (Amadi et al., 2015). Complementary to an apparent decrease in GABA after anodal stimulation, is evidence showing a decrease in glutamate concentration post-cathodal stimulation (Stagg et al., 2009). For anodal stimulation, it appears possible that the decrease in GABA concentration is the result of decreased glutamic acid decarboxylase (GAD) activity, as it has been shown in cats that anodal tDCS in the visual cortex reduces GAD(-67) (Patel et al., 2018). GAD, an enzyme that converts glutamate to GABA, may therefore be a possible mechanism for the after-effects of tDCS.

N-methyl-d-aspartate (NMDA) receptor sensitivity is also a possible mechanism by which tDCS' after-effects occur. Roche and colleagues note in their review that research has suggested NMDA receptor sensitivity is altered by tDCS (Roche et al., 2015). Together, the change in glutamate levels and the sensitivity change in the glutamate-agonized NMDA receptors seem to paint the picture of a possible mechanism for anodal and cathodal influence on post-stimulation excitability, where a decrease in GABA and increase in NMDA sensitivity would cooperatively contribute to cortical excitability, and a decrease in glutamate and reduced sensitivity of NMDA receptors would decrease excitability (Roche et al., 2015). Thus, the current understanding of motor task learning-modulation by tDCS is that by targeting the M1 or other motor-related

regions, mechanisms like increasing excitability and modulation of EPSPs allow for plasticity changes resulting in improvements in motor learning, both online and offline (Figure 2.4).

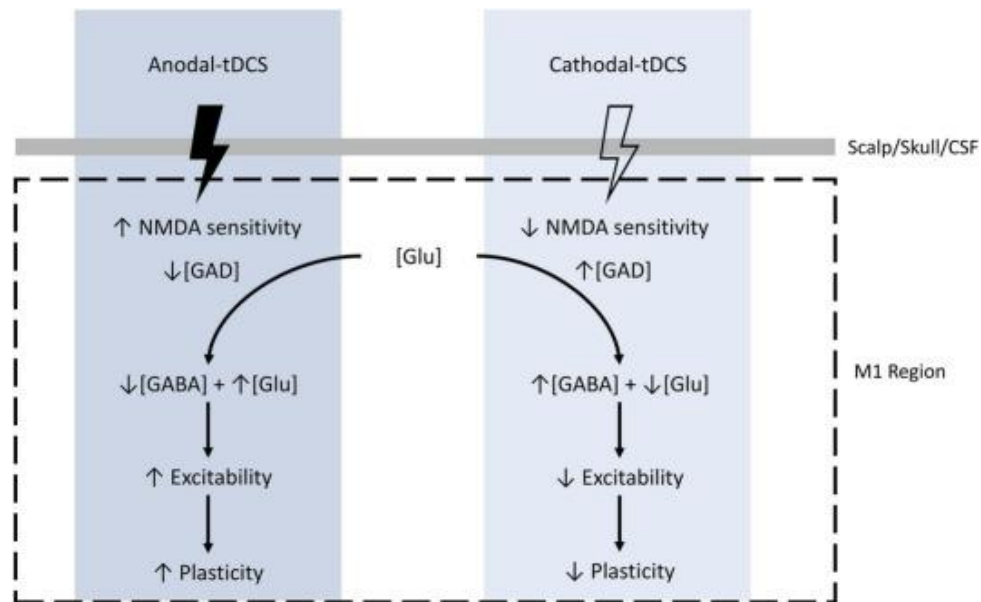


Figure 2.4 Anodal and Cathodal tDCS and underlying neurochemical mechanisms. Hypothetical model of a possible mechanism for a-tDCS/c-tDCS delivered to the M1 cortical neurons, resulting in plastic changes in motor learning. ([Glu] = concentration of Glutamate, [GABA] = concentration of Gamma-aminobutyric acid, NMDA = N-methyl-D-aspartate (increases or decreases in sensitivity)).

The effect of tDCS on motor retention refers to the improvement of how well the learned task is maintained over periods of non-practice. One study used dual-tDCS in a stroke population, it was found that tDCS resulted in improved performance during the online component, and improved long-term retention (1 week) (Lefebvre et al., 2013). This study employed 30 minutes of stimulation during the task training and began to see significant performance differences at the 15-minute mark. This is consistent with our proposed idea that some studies fail to see online improvements because the non-preconditioned task is too short to quantify the difference, which takes a variable amount

of time to begin inducing measurable plasticity changes and behavioural correlates. The improved performance was consistent for the remainder of the task, 30 minutes, 60 minutes, and 1 week after. In another study, it was reported that long-term retention of regained motor ability in individuals that suffered from stroke was better maintained 3-weeks after learning in a group that received dual-tDCS compared to those that did not (Goodwill et al., 2016).

Some adaptations of this approach, like one that used a combination of reward and tDCS delivered over the M1 region, were also successful in improving retention, finding that concurrent reward and current delivered during motor learning were effective, but neither one by themselves (Spampinato et al., 2019). In that study, the authors attribute the apparent synergistic effect to a joint use of long-term potentiation processes in both tDCS and reward. The authors also note that GABAergic synaptic modulation is a likely culprit for motor learning modulation, suggesting that reductions in M1 GABAergic inhibition lead to improved retention (Spampinato et al., 2019). Therefore, it seems that mechanistically, the after-effects of tDCS that act on consolidation, may similarly influence retention.

Conclusively, each of the acquisition, consolidation, and retention stages of motor learning has shown promise in motor stage learning modulation. However, when divided, it becomes apparent that each stage is distinct in the effect that tDCS elicits and the mechanisms that are believed to underly the effect. The variability in results between stages further supports the need for a stage-specific approach to tDCS and motor learning, since many studies report effects in 1 or more stages, but do not consider individual stage mechanisms in their discussion. While it may certainly be that mechanisms are shared between stages, there are likely distinct, but interacting mechanisms for acquisition, consolidation, and retention, which are often not approached as such.

TACS is applied in a similar manner to tDCS, with the only difference being the addition of a frequency in current delivery. In tACS, current is delivered in an alternating waveform with predetermined (or undetermined, in noise-delivered tACS) frequency. The appeal of tACS is the specification of the oscillatory aspect that can be used in delivery to achieve frequency-specific effects. Broadly, researchers are able to choose the delivery frequency of the stimulation, with the goal of inducing different effects based on the chosen frequency and region (Feurra et al., 2013). Such effects have included gamma-tACS (70 Hz) improvements of bimanual motor learning (Miyaguchi et al., 2022), alpha-tACS (10 Hz) for entrainment of cortical alpha waves, and beta-tACS (20 Hz) resulting in a movement speed reduction during motor task (Wach et al., 2013). This section will therefore discuss the application of tACS in motor task learning stages, the effects of varying stimulus frequencies, the underlying mechanisms, and the outcomes of tACS motor task modulation. Like tDCS, tACS for motor learning is typically delivered to the M1 or SMA region (Miyaguchi et al., 2022; Pollok et al., 2015b; Wach et al., 2013), although recently researchers have begun investigating cerebellar stimulation (Naro et al., 2017). tACS delivery can be applied anywhere from 0.1 Hz to 200 k Hz, and sometimes at no specific frequency at all, using frequency ‘noise’ in transcranial random noise stimulation (tRNS) (Antal & Paulus, 2013). Specific tACS effects on alpha waves, for example, have shown that tACS not only elicits phase entrainment of endogenous alpha waves, but also has effects on the power of the entrained oscillation (Helfrich et al., 2014). For further evidence of frequency and task specificity, (Feurra et al., 2013) presents results that show beta-tACS increases motor evoked potentials (MEP) *strictly* during rest when delivered to the motor cortex, while theta-tACS was effective during task state compared to dormancy. For implicit motor learning, low (40 Hz) gamma-tACS was shown to slow response times and decrease MEP amplitudes during an SRT task

when delivered over the left M1 region (Giustiniani et al., 2019a). This contrasts a higher-gamma study (Miyaguchi et al., 2022) which found the opposite, and further corroborating frequency specificity of tACS effects.

TACS acts differently to tDCS in that instead of inducing changes in electrical field to alter resting membrane potentials, its oscillatory component instead allows for entrainment of neural activity in numerous roles including classical alpha wave inhibition (Rumpf et al., 2019) and beta-band excitability (Yamaguchi et al., 2020). Specific to the motor cortex, tACS is believed to modulate motor excitability in the same way as anodal tDCS, however with frequency specificity being a determining factor in how long the effect lasts (Herrmann et al., 2013). Researchers must exhibit caution when theorizing the mechanism of tACS, since it is difficult to discern effects that result from stimulation (no different from tDCS), and those that result from the oscillatory aspect. In a similar fashion to tDCS-alterations of GABA, tACS has also been shown to alter GABA_A activity, which in turn predicted ability to learn a motor task (Nowak et al., 2017). Unlike tDCS, however, no alteration in cortical excitability was observed in that study, suggesting again that the effect related to oscillatory mechanisms, and not those of excitability. Like tDCS, we will approach the effect of tACS on motor task learning in reference to stages, aiming to provide evidence for the benefits a stage-specific approach.

In a study investigating tACS effect on motor learning, stimulation was delivered at 10 Hz, 20 Hz, and 35 Hz (Pollok et al., 2015b). This study found that during an SRT task, 20 Hz tACS over the M1 region during the task resulted in faster reaction times as compared to the sham stimulation. The authors state that 10 Hz and 20 Hz tACS effects were effective in modulating implicit motor learning, while 35 Hz was ineffective in modulating performance. Another study by Antal and others found that 15 Hz tACS delivery did not improve motor performance (Antal et al., 2008). For context, the 35 Hz

tACS delivery in Pollok et al., it was intended to act as a determinant of frequency specificity, which the authors suggest is therefore a similar effect in the 15 Hz (Pollok et al., 2015b). Since 15 Hz did not match the endogenous beta or alpha rhythms, it did not improve motor learning, similar to 35 Hz stimulation. These findings are suggestive that tACS can elicit frequency-specific acquisitional improvement. This provides specific options for neural modulation, and suggests an additional mechanism through the modulation of cortical oscillations using tACS (Helfrich et al., 2014). It must be considered then, that if 10 Hz and 20 Hz delivery improved motor learning, while 15 Hz and 35 Hz delivery did not; do the 10 Hz and 20 Hz frequencies work by the same or different mechanisms? To add to the complexity, another study by Wach et al., which delivered tACS at both 10 Hz and 20 Hz to M1, as did Pollock et al., found that while 10 Hz tACS increased movement variability, relating to irregularities in movement implementation, 20 Hz tACS produced movement slowing (Wach et al., 2013). Their study found that 20 Hz effects were evident immediately after tACS, while 10 Hz effects occurred at 30 minutes following stimulation. It is thus suggested that different stimulating frequencies induce behavioural effects in different time windows, and potentially by different mechanisms. Although Pollock et al. and Wach et al. both delivered tACS at 20 Hz, their results diverged. Importantly however, is that in Pollock et al., the results indicate behavioural changes *during* stimulation, while in Wach et al., the results are changes observed *following* stimulation. This is an important distinction, since the pre/during/post effects of tACS may elicit different behavioural effects.

Interestingly and consistent with the theory put forth in this review, Wach et al. also reported that, like tDCS-induced after effects, tACS-induced motor effects persisted up to 30 minutes post-stimulation (Wach et al., 2013). In the study by Pollok and others, it was determined that 10 and 20 Hz tACS facilitated learning of the implicit motor

sequence (Pollok et al., 2015b). The authors put forth the theory that tACS improves performance via acquisitional modulation by promoting synaptic plasticity through motor cortex excitation. The authors also draw attention to the findings that show 20 Hz stimulation was more effective in acquisition and go on to suggest that functional reorganization occurs because of stimulation, and improves not only acquisition, but also early consolidation. Similar effects have been replicated using gamma tACS (75 Hz) (Akkad et al., 2021). In their study, Akkad and colleagues used theta-amplitude-coupled gamma frequency tACS delivered to the M1 region during a thumb abduction task. The results found that the tACS group receiving ‘peak’ (strictly the positive component of the waveform) gamma tACS stimulation had better motor acquisition than the sham group. Research has also shown that beta-tACS (20 Hz) inhibited motor learning acquisition, while gamma-tACS (70 Hz) improved acquisition (Bologna et al., 2019). Again, studies have also found contrary results on the effect of gamma-tACS on motor acquisition, as it has been shown that gamma-tACS reduced performance improvement during an SRT task in only the last two blocks of the study (Giustiniani et al., 2019a). We find again that this delayed effect is evidence that tACS, like tDCS, may have a period of time before its effects are measurable in behaviour. This highlights the importance of a stage specific approach, since overlapping effects and after-effects can confound the actual behavioural correlates of the stimulation, and indeed their effects can differ accordingly.

The mechanism underlying the after-effects of tACS are not well understood, as is apparent by the numerous, and contradicting results. It is suggested in Wach et al. that there is a likely resonance effect on neuronal membrane potentials, and furthermore that increasing oscillatory activity may lead to disruptive neuron activity, which would provide a possible explanation for the effect of the 20 Hz slowing effect presented in their study (Wach et al., 2013). It does not however, explain why other studies report

improvement in motor learning at 20 Hz tACS, while delivering stimulation to the same brain region. One possible explanation is that the effect of tACS strengthens synaptic connections with similar resonance frequency to that being delivered, while weakening those with different resonance frequencies (Wach et al., 2013). If true, then the nature of the task would indeed be influential in the effectiveness of the stimulation. As for example, it has been shown that post-movement beta, parietal gamma, and prefrontal theta oscillations are associated with adaptive learning, with specific effects that include prefrontal theta influences on acquisition, and beta-related trial to trial adaptation (Struber et al., 2021). Therefore, since different motor tasks produce different endogenous rhythms (e.g., beta activity when learning a motor sequence, vs alpha activity when tracking a target), to truly compare the effect of one tACS study to another, the task, length, stimulus settings, and duration must be equivalent. This trend further highlights the need for not only stage-specific, but context-driven findings in tACS motor research. When considering the apparent inconsistent findings in tACS research, if we apply a stage specific, and furthermore task-specific context, the findings may no longer be termed contradictory, but rather highly specific.

Pollok and colleagues published an earlier paper that used magnetoencephalography (MEG) to understand oscillatory components of motor learning (Pollok et al., 2014). They present that, using an SRT-type task, changes in beta-band suppression were correlated with improved reaction times, which they attributed to the beta activity modulation as a marker of reorganization during motor learning and early consolidation. Combined, both studies by Pollok and colleagues (Pollok et al., 2015b; Pollok et al., 2014), suggest that tACS at the beta range can modulate both learning and early consolidation.

In further support for stage specificity, is the fact that research that delivered tACS during the consolidation stage (immediately after acquisition) has found little evidence that suggests motor consolidation is improved by tACS (Roshchupkina et al., 2020). In their study, Roshchupkina et al. used beta-tACS to modulate motor task learning, delivering stimulation after the initial learning session, and then at two time points after learning (25 minutes, and 4 hours). Although preliminary, this finding is potentially supportive of the earlier observation in this review, which is that some research fails to find modulatory effect during the stage of stimulus delivery, because the effect on the brain may occur after stimulation has ceased, and is therefore misaligned with motor learning stage. As mentioned, this effect has been seen in tACS already, with an approximately 30 minute after-effect (Wach et al., 2013). In a different approach, Rumpf and colleagues delivered 10 and 20 Hz tACS to an elderly population and found that alpha (10 Hz) tACS delivered post-training interrupted consolidation or retention, resulting in poorer performance in testing (Rumpf et al., 2019). Interestingly, the authors suggest that these findings are not the result of modulations of cortical excitability, but instead attribute the behavioural changes to the inhibitory nature of alpha rhythm. It is suggested that the endogenous alpha activity is entrained and enhanced with delivery of alpha tACS, resulting in inhibition of consolidation. This finding is relevant because it supports that the effect of modulation is at least partially elicited via the oscillatory component in delivery, and not strictly the current intensity. Conclusively, the placement of stimulation in the task paradigm is apparently critical for interpreting findings, particularly when employing tACS, since there is the introduction of another variable (frequency) which introduces new mechanistic theories.

Akkad et al. found that retention was also better in the tACS group compared to the sham group, with replicable results (Akkad et al., 2021). It is also reported that tACS

delivered in the beta range after the acquisition stage showed improvements in performance at both 1 and 7 days post-practice (Yamaguchi et al., 2020). This finding is potentially the result of beta-band induced excitability, which is suggested to enhance retention of motor memory. The authors also note, however, that this may be the result of improved consolidation of the motor task. This is an important point, as it suggests that measurements of retention are also likely measuring a component of consolidation- since it is arguable that for a motor memory to be retained in the long-term, it must be effectively consolidated. In further support of a beta-band effect on retention, are studies that illustrate beta-tACS improved retention up to at least 7 days after acquisition (Yamaguchi et al., 2020), and furthermore that gamma-tACS improved retention 24 hours after acquisition (Miyaguchi et al., 2020).

In support of the after-effect hypothesis, the study by Miyaguchi et al. did not find acquisitional differences, and only reported significant retention improvement in the tACS group, although it is important to note that Miyaguchi et al. delivered stimulus at both M1 and the cerebellum. In the previous paper mentioned by Giustiniani et al. (Giustiniani et al., 2019a), it was reported that performance was disrupted by gamma-tACS delivered over M1. The authors attribute this trend to an impairment of retrieval of the learned SRT sequence. In another study, Giustiniani et al. delivered gamma-tACS instead to the cerebellum, and indeed a similar interference effect was seen, however as the authors note, in acquisition of the sequence, and not in retrieval (Giustiniani et al., 2021). The authors note that these findings are consistent with others that find that tDCS studies show contributions to acquisition by the cerebellum, and retention by the M1.

It is noted that the tACS mechanism underlying this retention improvement is likely related to oscillatory entrainment, as it is shown that for example, gamma band activity has been shown to enhance neural network activity between varying cortical

regions, and thus may improve performance (Lee et al., 2003). In the study by Yamaguchi et al., tACS was delivered over M1 alone, and the authors suggest that the effect on retention may be the result of an induced long-term potentiation resultant from beta band activity, which has been shown in animal models (Yamaguchi et al., 2020). Furthermore, in reference to the apparent inconsistency in results between the effect of gamma-tACS on retention and acquisition, there are numerous explanations presented by Giustiniani et al (Giustiniani et al., 2021). Firstly, that by modulating Purkinje cells in the cerebellar cortex, long-term depression necessary for plasticity may have been prevented, secondly, that the endogenous rhythms of the Purkinje cells was optimal during the task, and that tACS introduced interference that resulted in deteriorated performance, and third, that components of the gamma frequency in the cerebellum were entrained, but that those components were not optimally modulated, or that the component modulated was not contributory to performance or plasticity improvement. The study also failed to find differences in corticospinal excitability, leading to the hypothesis that gamma-tACS impaired cerebellar contributions to motor learning, while unaffected M1 excitability.

In support of stage specificity, is that many of the studies presented here often find differing effects dependent on the stage placement of stimulation. Studies report that the same stimulation delivered during acquisition elicits no changes, while during retention can improve performance. Indeed the large amount of stimulation variation, task paradigms, and potential mechanisms suggests the need for consistency where possible.

Therefore, we find that although there are what appear to be growing inconsistencies in tACS literature surrounding the effect of specific frequencies, further complicated by the variation in location of delivery, we agree with the claim that this apparent inconsistency is simply the result of high specificity. Thus, it appears beneficial

to categorize tACS by the motor stage under modulation, to allow for discrete categorization of effect, and to guide hypothesizing.

2.4 Peripheral nerve stimulation and motor learning

There are natural criticisms that arise when using tDCS and tACS to modulate motor learning. Despite our attempt to produce clarity in the variability in results of tDCS and tACS studies, the fact remains that our efforts are guided by the present literature available to us, and as such further research is necessary to better understand the mechanisms of tDCS and tACS. The evidence presented here aims to provide sufficient basis to convince the reader that by approaching brain stimulation of motor learning with stage-specificity, that further research aiming to uncover the mechanisms underlying the effects will be more applicable and specific. Further research, however, may find that stages coalesce in mechanism, or that our staging is oversimplistic. We therefore offer these recommendations as exactly that.

Another caveat to much of the motor research being done using tDCS is that most studies indicate that the effects seen, both mechanistically and behaviourally, are the result of non-invasive stimulation of the brain itself. Recently however, research has investigated the necessary parameters needed to induce the local effects described. Research using a cadaver approach found that nearly 6 mA of current are needed to effectively manipulate brain network activity through scalp-applied electrodes, with a nearly 66% attenuated current by scalp and skull (Vöröslakos et al., 2018). This is counterintuitive, considering that many motor task studies using on-scalp electrodes, such as tDCS, typically apply current in the range of between 1-2 mA (Bastani & Jaberzadeh, 2012; Buch et al., 2017; Jo et al., 2009; Khedr et al., 2013; Wiltshire & Watkins, 2020). This implies that there may be more than a localized brain-stimulation effect involved when modulating activity via scalp-electrode stimulation. tACS also

raises questions in its mechanism of action in the same way as tDCS, with typical delivery voltages falling below the 6 mA level mentioned (Vöröslakos et al., 2018). Of course, a valid response to the findings of that study is that delivery of electrical current to cadaver compared to live humans is likely a confounding aspect of the research. This is valid criticism; however, it also does not rule out the possibility that peripheral nerves play a role. This therefore leads us to ponder how stimulation directed toward central regions, may actually or additionally act by peripheral mechanisms, and additionally to what extent this mechanism may be prevalent in the studies previously mentioned here, in both tDCS, and tACS.

Despite the large collection of studies and numerous proposed mechanisms, there is still a relatively low understanding of the exact mechanism by which tDCS and tACS function. This concern is highlighted by recent studies whereby it is discussed that typical current strengths applied to the scalp do not appear to maintain a significant current for neural entrainment after attenuation by tissues (Asamoah et al., 2019; Vöröslakos et al., 2018). Work by Huang and colleagues also offers an *in vivo* observation of electrical stimulation effects on the brain, finding similar attenuation of current to below physiologically significant levels for certain neural effects (Huang et al., 2017). There is a suggested solution to this so-called paradox occurring in the literature. Instead of being stimulated directly through the scalp, the observed neural entrainment by tACS (Asamoah et al., 2019) and activation by tDCS (van Boekholdt et al., 2021) may indeed either be partially or entirely accountable to the stimulation of peripheral nerves, which form ascending connections to the brain. The more recent suggestion that many of the effects seen in both tDCS and tACS studies may in fact be peripherally modulated is becoming more understood as researchers begin to test the effects of peripheral nerve stimulation on previously direct-stimulation effects. Up to now, disciplines such as

memory (Luckey, McLeod, Mohan, & Vanneste, 2022), motor system modulation (Asamoah et al., 2019), tinnitus treatment (De Ridder et al., 2014), plasticity and reorganization (Daniel R Hulsey et al., 2016; Loerwald et al., 2018), and stroke recovery (Dawson et al., 2016) have been reproduced with peripheral approaches instead of direct stimulation. To improve concision and understanding, we put forth alternative definitions for peripheral stimulation, such that tDCS and tACS delivered peripherally will be referred to in relation to the nerve under stimulation (e.g., Vagus nerve stimulation (VNS), Occipital nerve stimulation (ONS)). One such acronym that will be used in this section is Non-Invasive Transcutaneous Electrical Stimulation of the Greater Occipital Nerve (NITESGON), an approach which is currently under active investigation at the time this thesis is being written (Adcock et al., 2022; Luckey, McLeod, Huang, et al., 2022). NITESGON involves the use of a typical tACS set-up, however with placement of the electrodes over greater occipital nerve. This arrangement has also been validated during an experiment in which a topical anaesthetic was used to block the transcutaneous mechanism (and verify NITESGON elicits its effect via peripheral effect and not transcranially). Indeed it was found that blocking the peripheral mechanism using lidocaine altered memory task performance compared to stimulation without lidocaine, providing evidence that NITESGON is effectively acting peripherally and not eliciting its effects transcranially (Vanneste et al., 2020).

To test the validity of the hypothesis that peripheral nerve stimulation is an potential contributor for the tDCS/tACS effects seen in motor learning, we turn toward the results presented in a paper by Asamoah et al. (Asamoah et al., 2019). Their findings used an encompassing approach to determine if tACS can induce effects by acting peripherally. They found that entrainment of the rat motor cortex was possible via peripheral stimulation, and in order to confirm this, used topical anaesthetic to block

peripheral delivery when delivering tACS. In this instance, resulting in reduced entrainment suggesting that tACS indeed had peripheral influence in rats. The next step was to test the same finding in humans, and the results were consistent in that tACS peripherally delivered appears to be, at least a component, of the driving mechanism behind the motor cortex entrainment.

The next question is therefore *how* does stimulation delivered peripherally account for at least a partial contribution to the modulation of the brain during motor task learning? We offer here some possible mechanisms by which peripheral delivery of stimulus may modulate neural activity in motor task learning. We also delve into how these mechanisms and the concatenation of the mechanisms in this chapter may function cooperatively to holistically explain the findings of numerous research articles.

In direct comparison of transcranial to transcutaneous current delivery mechanisms, we first consider the biological and tissue differences. Transcranial delivery involves current that is passing through layers of skin, skull, cerebrospinal fluid (CSF), and altering activity either via oscillatory entrainment or polarity of the cortical neurons, or perhaps even deeper as is put forth by (Louviot et al., 2022). Transcutaneous stimulation, in name, does not involve current passing current through CSF or skull (which leads to current attenuation) (van Boekholdt et al., 2021). It is believed that the greater occipital and trigeminal nerve can be stimulated via peripheral stimulation, and these nerves then may functionally modulate activity in the nucleus of the solitary tract (NTS) and trigeminal nuclei (TN) (Adair et al., 2020; van Boekholdt et al., 2021). The TN is a component of the trigeminocervical complex (TCC), and has ascending connections through the quintothalamic tract, to the thalamus which further relays to the cortex, suggesting a possible motor cortex modulatory pathway for peripheral stimulation (Akerman et al., 2011). It has also been shown that the NTS has projections to the locus

coeruleus (LC) (van Boekholdt et al., 2021). This is a relevant point, as the LC is accountable for the release of norepinephrine and thus subsequent cortical excitability, of which tDCS has been implicated (increases of NE) (Hsiao-I Kuo et al., 2017). This however, is yet unexplainable with sound mechanistic support (van Boekholdt et al., 2021). When using VNS for treatment of epilepsy, the LC is considered to be a component of the mechanism behind the central effects (Krahl & Clark, 2012). More direct evidence has also been demonstrated, in a recent paper by Liebe and colleagues (Liebe et al., 2022), which found indeed that among numerous LC functional and anatomical connections, that the LC has a positive functional connectivity with the motor cortex, specifically parcellation 6ma, which posits further evidence for LC-driven connectivity being a feasible mechanism for NITESGON and other peripheral nerve stimulation (e.g., VNS). Using VNS in rats, a study by Hulsey and others showed that stimulation induces intensity-specific activity of LC neurons (Hulsey et al., 2017). Agreeing further with the proposed pathways of activation. The same study (Hulsey et al., 2017) found that trigeminal nuclei neurons were also activated, and phase locked, to the VNS delivered. Indeed it has been shown that the occipital nerves (ON) exhibit function connection to neurons in the TCC, and furthermore that NMDA receptor antagonization reduced the responses in the TCC (García-Magro et al., 2020). This provides more context for the involvement on NMDA receptors in peripheral nerve stimulation and draws comparisons in the mechanisms of central and peripheral stimulation.

Further VNS investigations using rats have also revealed likely neurotransmitters involved in neural modulation via peripheral stimulation. In a report by Hulsey and colleagues (Hulsey et al., 2019), the hypothesis that norepinephrine (NE) and serotonin (5-HT) are involved in motor cortex plasticity changes with VNS was investigated. It was

determined that VNS induced motor cortex plasticity changes however only with the presence of either NE or 5-HT. In another study (Daniel R. Hulse et al., 2016), it was also determined that cholinergic projections are essential in inducing VNS-motor cortex reorganization. These studies create strong evidence that NE, 5-HT, and cholinergic projections are involved in the mechanism for VNS-induced motor plasticity. Since VNS and ONS have similar functional connectivity, it may be plausible that similar neurotransmitters may be implicated. This is particularly relevant, as the same findings of serotonin (Nitsche et al., 2009), NE (Adelhöfer et al., 2019), and cholinergic system (Oh et al., 2022) effects have been presented in tDCS and tACS studies.

The review by van Boekholdt et al. also draws attention to the similarity between cortical and peripheral anatomical similarities that posit cortical anodal and cathodal differences are persistent in peripheral nerve stimulation (van Boekholdt et al., 2021). This produces further explanation for the polarity-specific effects seen in previously discussed studies (Bastani & Jaberzadeh, 2012; Brunoni et al., 2012; Sasaki et al., 2016). To organize and strongly recommend the idea that tDCS/tACS may act peripherally instead of/as well as centrally, the effects and mechanisms proposed in tDCS and tACS must have a parsimonious explanation in their peripheral mechanism (Figure 2.5). It has been accounted for in this chapter that polarity-specific effects shown in studies can be accounted for in peripheral nerve stimulation, and that commonplace neurotransmitter effects such as 5-HT and NE have presence in both central and peripheral stimulation. However, other observed correlates of CS such as GABA/Glutamate alteration, oscillatory entrainment, frequency-specificity, and delivery-location effects require peripheral equivalents. Research using VNS for epileptic patients has found that GABA receptor density (GRD) decreased after VNS, subsequently resulting in a reduction in seizure frequency, compared to an unchanged frequency and GRD distribution in the

control, unstimulated, group (Marrosu et al., 2003). Memory improvements in both rats and humans as the result of VNS are attributed to processes involving the excitation of the hippocampus and associated neural networks; specifically with the presence of theta rhythm (Boyce et al., 2016; Ura et al., 2013). Research by Broncel et al. (2019) has shown that GABAergic receptors are instrumental in producing theta rhythm, while in cooperation with cholinergic systems, and that VNS stimulation results in modulation of GABAergic processes, and as such produces a possible candidate mechanism that further implicates peripheral nerve stimulation on GABA-related processes. We return to the study by Asamoah and colleagues (Asamoah et al., 2019) to compare oscillatory entrainment in peripheral vs central stimulation delivery. In their paper, they show that transcutaneous nerve stimulation resulted in entrained beta activity as recorded via electroencephalographic recording (EEG), although the exact mechanism by which peripheral tACS elicits this entrainment was not explicitly discussed. This is suggestive that oscillatory entrainment has been shown to occur via peripheral stimulation, supporting the contributions of a peripheral mechanism.

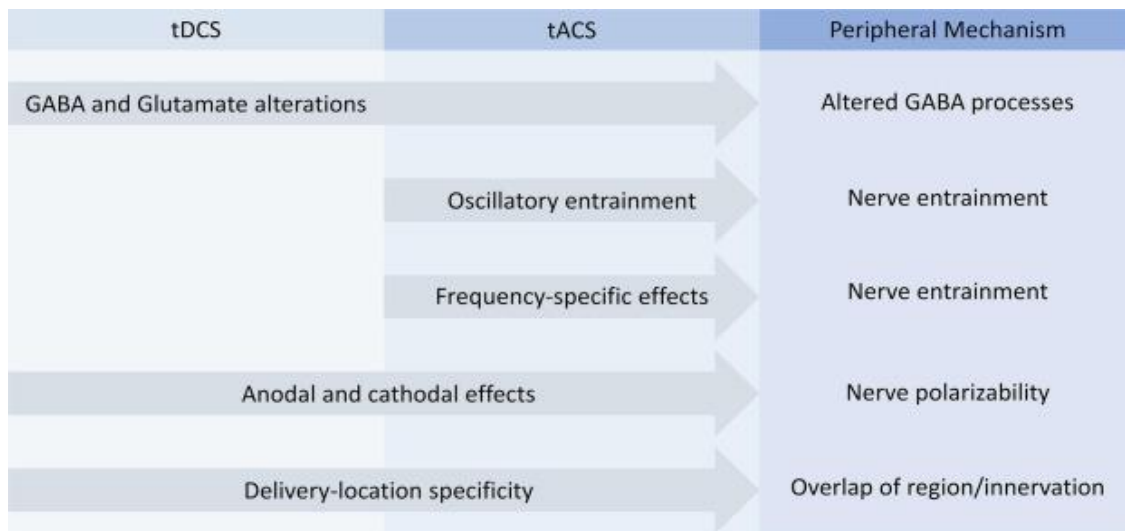


Figure 2.5 TDCS and tACS mechanisms and corresponding peripheral explanations. An illustration that shows how each mechanism for either tDCS or tACS has a plausible peripheral mechanism which may produce a similar result. For tDCS, GABA and Glutamate alterations exist in peripheral GABA processes. In tACS, oscillatory and frequency-related effects have been shown to result from nerve entrainment. For both tDCS and tACS, anodal and cathodal difference and location-specific activation may be explained or supported by nerve polarizability and regional innervation of peripheral nerves.

The last aspect of the stimulation mechanism which persists the possibility that tACS and tDCS may have some central/direct delivery component, is location specificity. Motor studies using brain stimulation to modulate motor learning typically deliver stimulus at the M1 region, although as mentioned, there are numerous other targets which are sometimes chosen. This raises the question of whether innervation of the scalp correctly correlates with the locations chosen in motor studies, such that peripheral nerve stimulation is still a viable mechanism? It appears that nerves such as the ON do indeed innervate the scalp as far as the vertex, which would corroborate the possibility that M1 stimulation does indeed stimulate the ON, while stimulating the cortical region directly (Yu & Wang, 2021). Further possibility is that cerebellar tDCS or tACS is also spatially similar to occipital nerve stimulation (ONS) study placement, creating the possibility that peripheral nerve stimulation is an additional contributor to the effects.

A study that investigated the central effects of occipital nerve (ON) stimulation using functional magnetic resonance imaging (fMRI) provides more evidence for this claim, showing that ONS both increased and decreased activity various areas of the brain, with decreased activity in the auditory cortex, visual cortex, somatosensory cortex, and amygdala, and increased activity in the thalamus, frontal, parietal, and cerebellar regions (Kovacs et al., 2011; Naro et al., 2017). As mentioned, the analysis in an article by Kovacs and colleagues suggested frequency dependency effects during nerve stimulation, which mirrors the frequency specificity cited in tACS studies mentioned earlier in this paper (Kovacs et al., 2011).

2.5 Future directions and criticisms

It is presented here that peripheral nerve stimulation causes widespread-activation, with frequency and location dependency, reflecting polarity-sensitivity, and with preliminary evidence of neurochemical similarities between central and peripheral delivery. There then appears a growing body of evidence to suggest that many studies using tDCS and tACS for delivery of central stimulation during motor task learning modulation, may be acting by an unconsidered and understudied mechanism. We therefore suggest that as research continues to further the understanding of the mechanisms of tDCS and tACS in motor modulation that there be careful consideration for the possibility of peripheral stimulatory effects and mechanisms of action, and indeed consideration for the motor learning stage and parameters which are chosen. It is additionally worth noting that much of the literature on peripheral nerve stimulation cited in this section was provided in the scope of epilepsy, migraine, tinnitus, and motor studies in animals. It may be considered that future research aims to fill gaps that obscure key understanding of how both central and peripheral nerve stimulation work. We highlight

some key points here in order to support researchers in identifying where research can be directed.

- 1) Necessity to further investigate the mechanism of action of peripheral and central nerve stimulation including the vagus, occipital, and trigeminal nerves, and a suggestion for researchers to investigate the distinction of transcranial from transcutaneous stimulation via methodologies such as those presented in (Asamoah et al., 2019).
- 2) The need to clearly outline target motor learning stage, and outcome in a stage-specific manner, such that mechanisms, interpretations, and reproducibility are streamlined and operationalized.
- 3) The importance of consideration for motor task used, electrode placement, and duration when reproducing or comparing studies, in order to reduce the apparent inconsistency in the field, and to allow for relevant comparisons to be made between findings.

2.6 Conclusion

We present here a brief overview of motor task learning, the stages of motor learning, common experimental approaches, and key features and anatomy of motor learning. With this background, we further explored the use of tDCS and tACS for manipulating motor task learning, including a specific focus on the stage of delivery, and the outcomes of numerous studies. The results of the studies and their findings led to an additionally, underlying mechanism of motor task modulation; that peripheral nerve stimulation may be acting to functionally modulate the motor cortices. We presented further evidence for this possibility, including accounting for central mechanisms in the peripheral domain and preliminary data showing effectiveness of peripheral nerve

stimulation. We conclude with recommendations for additional consideration for a peripheral mechanism. It is our aim in this review to provide an overview of motor task modulation by stage, via tDCS and tACS – and in the hopes that future research may be able to refine approaches with greater specificity and context.

Chapter 3 The Neural Correlates of Online and Offline Motor Learning²

3.1 Introduction

Brain imaging research has been used to uncover the neurobiological underpinnings of numerous cognitive functions and processes. When performed alongside a task, imaging allows researchers to visualize the activity of the brain, correlating activity with function and process. During motor tasks, this method uncovers the activation changes and processes that underly the domains of movement, working memory, error detection, adaptative learning, and attention (Seidler et al., 2012; Shadmehr et al., 2010).

Specific to motor learning, there are both continuous and transient neural processes which govern how the brain learns movements (Verhoeven & Newell, 2018). Although there is variability in the naming of the processes, there is consensus that motor learning begins with acquisition (sometimes called learning or motor adaption), which is followed by consolidation, and then a longer and less defined stage in which the brain retains the learned skill, sometimes referred to as retention (Luft & Buitrago, 2005). At the behavioural level, acquisition is the initial learning of the task, either during (fast) or between sessions (slow) (Luft & Buitrago, 2005), or alternatively described as occurring over three phases, the cognitive, the associative, and the autonomous (Marinelli et al., 2017). Functionally, motor learning acquisition has been correlated with activation of the primary motor regions, sensory regions, cerebellum, basal ganglia, prefrontal cortices, cingulate cortices, and parietal cortices, dependent upon the type of task being used (Ghilardi et al., 2000). In the domain of adaptive motor learning, acquisition manifests as the combined influence of an internal forward model, continued adaptation to motor targets, and the integration of information from commands and its subsequent sensory feedback (Shadmehr et al., 2010). Such a system relies on the same regions which have previously been associated

² The following chapter incorporates material which is currently under review in *Imaging Neuroscience*.

The author of this thesis is the first author on the manuscript.

with motor learning. Specific systems including the cortico-striatal and cortico-cerebellar are implicated as being essential for acquisition and performance of learned motor skills (Doyon & Benali, 2005), albeit there is no meta-analysis to our knowledge that has assessed the consistency of activation of the cortico-striatal network across multiple forms of motor learning experiments, as it pertains to the adaptive motor learning framework.

In *consolidation*, the stage which follows the initial learning of the task, the learned skill is consolidated (however is susceptible to interference). It has been illustrated that the M1 region is involved in motor consolidation, with one study showing that brain activity shifts from prefrontal to premotor, parietal, and cerebellar regions (Muellbacher et al., 2002). Research has implicated other consolidation-related structures including the striatum, somatosensory cortex, lobule VI of the cerebellum, and the caudate nucleus (Debas et al., 2010; Doyon & Benali, 2005; Wali, 2020). Following acquisition via adaptive processes, the newly acquired internal model becomes increasingly consolidated and thus resistant to interference by a competing model (Shadmehr & Brashers-Krug, 1997).

Accordingly, motor learning acquisition (via the processes of adaptation and development of the internal model) is a form of online learning, while learning effects during motor consolidation would be considered offline learning (whereby the internal model becomes increasingly resistant to retrograde interference). It has been shown that these forms of learning are governed in some ways by similar regions (Ehsani et al., 2016), and in other instances, different ones (Lacroix et al., 2019; Pollok et al., 2020). A pattern then emerges where regional activation changes across motor learning processes, albeit not necessarily for the same reasons. One consistent network which appears engaged in both online and offline motor learning is the cerebral network, which includes the M1, pre-motor cortex, supplementary motor area (SMA), basal ganglia, prefrontal cortex, posterior parietal cortex, and cerebellar regions (See Figure 1 in Dahms et al. (2020) for a clear schematic of how this network is engaged over motor learning stages).

Although there is extensive work in motor learning, with a strong understanding of the brain activation patterns that occur during different timepoint in the learning process, there are

few studies which broadly discuss the neural correlates of online and offline learning, as they relate to motor learning, and the current theories surrounding their mechanisms. One known review (Di Rienzo et al., 2016) investigated online and offline performance gains during motor imagery, as it relates to the promotion of motor learning. They conclude that online and offline learning contribute to performance, highlighting that the neurophysiological correlates of offline motor imagery practice are generally unexplored. We therefore feel it important to produce a similar analysis which investigates online and offline motor learning correlates across previous motor learning studies, in order to validate and judge the consistency of activation across a heterogeneous literature of task choices, demographics, and scanning points. This meta-analysis would then seek to relate the neural signatures to current frameworks in motor learning in order not only to support regional activation, but relate online and offline differences in the context of current motor learning theory (Gupta & Rickard, 2024; Shadmehr et al., 2010).

We believe that it is valuable to create a generalized picture of a theoretical motor learning experiment where the online and offline correlates of motor learning can be effectively compared and contrasted using meta-analytical methods. By performing a coordinate-based meta-analysis (CBMA) on studies that looked at different periods of motor learning, we can create a binary representation of motor learning across online and offline stages that may better capture the transient or persisting nature of specific activation patterns and processes. Functional-imaging meta-analyses are becoming increasingly popular, additionally with the support of large-scale neuroimaging databases such as NeuroSynth (Yarkoni et al., 2011) and NeuroQuery (Dockès et al., 2020). Indeed, meta-analytical environments such as NiMARE (Salo et al., 2023) further organize and simplify the meta-analytical process, by combining numerous tools and databases into a single library and allowing users to access a larger scale of resources to complete rigorous and reproducible analyses. The objective of this study is therefore to use a coordinate-based meta-analysis with activated-likelihood estimate (CBMA ALE) approach to investigate motor learning studies available on the NeuroQuery, NeuroSynth and PubMed databases.

In the following meta-analysis, we aim to produce an estimated representation of how brain activation patterns during two aspects of motor learning (online and offline) may transition

or persist, which will provide context and clarity to how regional activation/deactivation may occur during motor learning. We will follow an approach that categorizes studies based on this criteria, and investigates unique and overlapping patterns between online and offline learning. This work intends to address the manifestation of neural signatures representing online and offline learning, allowing for a clear comparison between the regions implicated, across a variety of motor learning tasks and paradigms. Identifying consistent regional activation will also test current hypothesis surrounding the circuitry involved in acquisition and consolidation, and place regional activation signatures in the context of online and offline motor learning theories, and their synthesis with adaptive motor control (Gupta & Rickard, 2024; Shadmehr et al., 2010). Accordingly, our results aim to be a generalizable representation of motor learning correlates during two critical periods in the process. We also aim to give context to the growing number of studies which investigate motor learning, and even stimulation applications (e.g., tDCS, tACS, TMS) to modulate motor learning, in order to provide insight into regional specificity in both online and offline targets for modulation, regardless of the task.

3.2 Methods

This meta-analysis sought to estimate activation changes across a two motor learning processes, by taking existing motor learning stages, and comparing and contrasting activation patterns between the scanning points. We chose to subdivide motor learning into either online or offline timepoints (either scanning during the task, or after the fact). Accordingly, we use the scanning time point as the categorizing factor. Scanning concurrent with the task learning would capture signatures of online learning (e.g., acquisition) or reflect neural processes of motor task performance, and scanning after the task or during rest would capture offline learning or the after-effects of the learning (e.g., consolidation or retention). We also opted not to reduce the study by criteria by the task under investigation, in order to 1) generalize our findings to motor learning processes non-specific to a given task, and 2) to allow more diverse study inclusion.

3.2.1 Study inclusion

The pipeline of study inclusion criteria is illustrated in Figure 3.1. All studies included had publication year, authors, title, and reported activation/deactivation peaks and coordinates listed. Studies were only accepted if they could be found online (e.g., internet search, database search). All studies included were in English. Studies included in the meta-analysis were required to have a clear study design, and have defined when the imaging took place in the course of the experiment. This was necessary to confirm what the reported peaks reflected (e.g., during or after task). Pathology groups or medication groups were excluded to ensure that coordinates reported were the result of motor task learning, and not the result of impaired or altered motor learning. In addition, studies with age extremes (i.e., less than 18) or studies that explicitly investigated ‘aging effect’ were not included, as activation changes due to aging or maturation may not represent motor learning in a comparable way (Zapparoli et al., 2022). Studies must also have a clear motor learning component (i.e., studies purposefully investigating motor tasks). Studies that included motor tasks without any learning aspect were excluded, since peaks reported in these studies may not reflect a learning effect. Studies included were required to be research articles, so reviews and meta-analyses were not included. Studies that were beyond the scope of the meta-analysis (e.g., non-human, ROI, investigations of subject variability) were excluded in order to refine the analysis to group-level activity reflecting motor learning. During extraction, studies were excluded if data was missing (i.e., peak value, coordinate values, cluster size), or if the data was of an incompatible type for a coordinate-based meta analysis (i.e., T-score instead of Z-score).

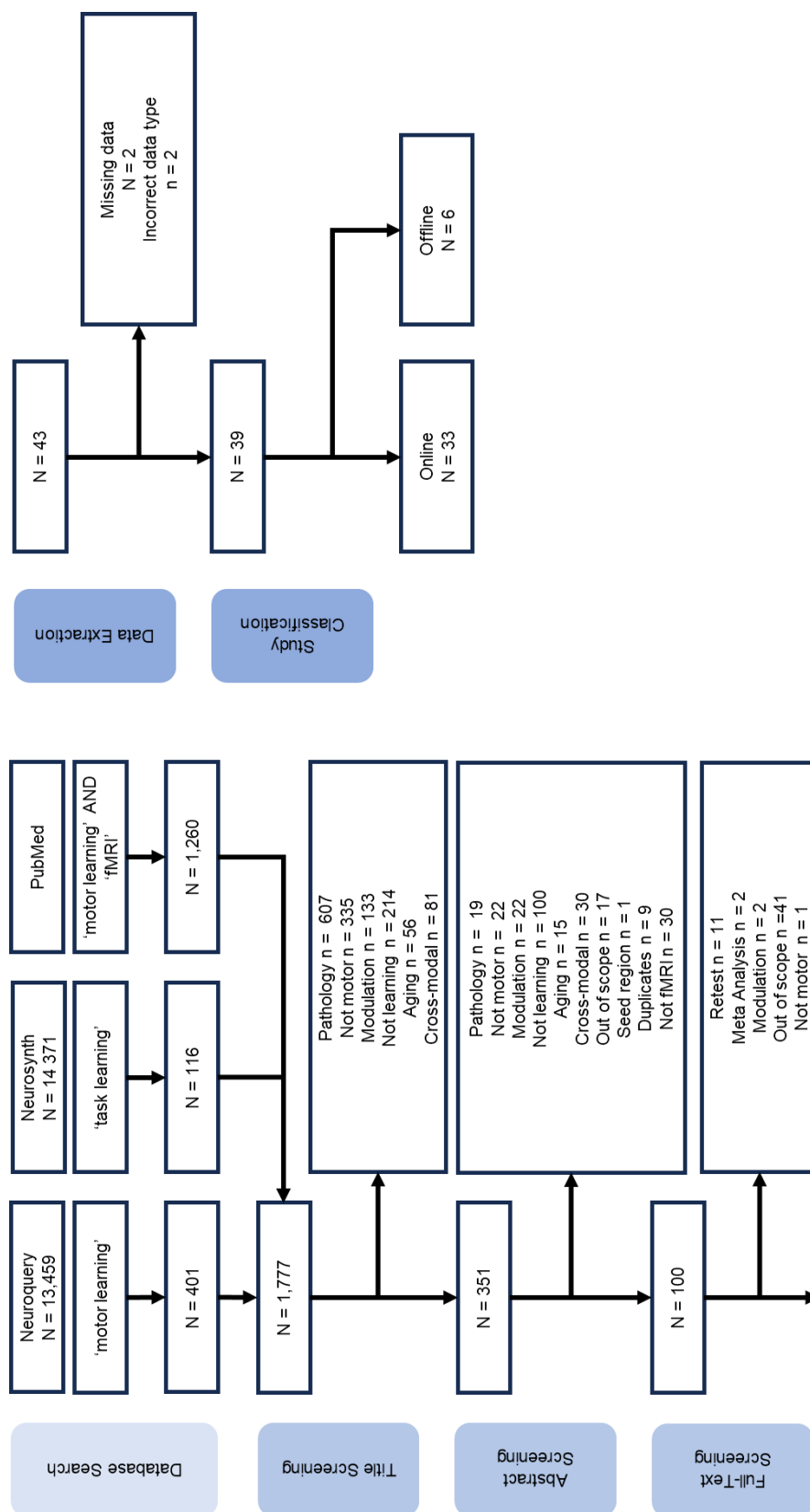


Figure 3.1 Pipeline of study inclusion from database search, title screening, abstract screening, full text screening, and categorization. Values for exclusions and inclusions are separated by database searched. Initial database search was automated, all screening and categorization were manual.

3.2.2 Database and search

The databases, NeuroSynth (<https://neurosynth.org/>) (Yarkoni et al., 2011) and NeuroQuery (<https://NeuroQuery.org/>) (Dockès et al., 2020) were searched through the NiMARE python package (NiMARE v0.0.11 (RRID:SCR_017398; (Salo et al., 2023))). PubMed was used to supplement studies which may not have been indexed by either database, in order to a more robust search methodology which is not restricted by the imaging databases.

In NeuroQuery, the abstract search term ‘motor learning’ was used. Following the initial search, to widen the search results using a varied search term, the NeuroSynth database was queried using the term ‘motor task’. The search terms differ because, while this meta-analysis intends to focus on motor learning, NeuroSynth did not support motor learning as a search term. It is expected that its use as a search term will result in a larger number of rejections for the dataset returned, since the search term was less specific to motor learning. The search term was limited to English, since to our knowledge the NeuroQuery and NeuroSynth databases only contain studies written in English. For more information about the NeuroQuery database, and its comparison to NeuroSynth as a meta-analytical search software, see the paper by Dockès and colleagues (Dockès et al., 2020). PubMed was therefore used to search studies which may not have been indexed within either NeuroQuery or NeuroSynth, but which may meet criteria.

All databases were searched in March, 2024. The initial database search was automated, and following the initial query, a manual selection process was carried out in two stages; 1) to determine inclusion or exclusion of each study, and 2) to categorize the included studies. At both stages, three independent reviewers screened each study for the inclusion/exclusion criteria. The title, abstract, and then full text of each paper were subsequently used to verify no clear exclusion factors, and study inclusion was then verified with the body of the text. If both reviewers agreed on the decision to include the study, it was passed to the second stage of categorization. Disputes were moderated by a third party. If all reviewers agreed that the study did not meet inclusion criteria, or had an exclusion criterion, then the study was rejected. In stage two, the same process was carried out, where two independent reviewers use the categorization criteria to determine

which category the study was to be included. If there was disagreement in decision, the study was given to the third independent reviewer, who moderated the decision on categorization.

3.2.3 Study data collection

Two reviewers independently gathered data from each report, and disagreements were handled with a third independent reviewer. All data was validated by a third reviewer. The number of peaks and their coordinates from each study were automatically derived using the NiMARE package, and all other study information was sourced from the literature. Due to the nature of this meta-analysis, the peaks and their coordinates are designated as the main outcome variable from the studies analyzed.

Data included the year of publication, the sample size, and type of task used, was reported, along with mean age, age range, and right handedness, and number of females in each sample (as available). When studies did not report a value, it was left blank and not inferred. In the instance where a study stated the initial sample demographic, but then reported withdrawals/data exclusions without updated demographic information, the corresponding demographic information was not inferred, and instead was left blank.

3.2.4 Synthesis and analysis method

An activation likelihood estimate algorithm was used to perform a CBMA on the studies. Briefly, an ALE analysis models the activation based on coordinates and peak values, for each study individually. The created activation maps are combined to a single map based on a voxel-based assignment of each ALE value, and compared to a null distribution, returning a z-map of the activation for each group/subgroup. It is important here to note that the data presented here does not strictly represent activation, but rather the coordinates and peaks reported are simply values at which there was significant increase or decrease in activation. Thus, while the ALE describes, in name, ‘activation’; the results represent the implication of regions in general - rather than *increased* activation. In an ALE analysis, a Gaussian kernel is used to account for the spatial uncertainty of the peaks by smoothing the values across neighbouring voxels. The width of the kernel is proportional to the sample size of the study, accounting for the likelihood that studies

with larger sample sizes are a better indicator of the true location of a peak (Eickhoff et al., 2009). The ALE analysis was performed on each group (i.e., online and offline), with an uncorrected significance threshold of $p < 0.001$, following previous ALE papers (Grosbras et al., 2012; Héту et al., 2013). Cluster volume for both ALE analyses was set to a minimum of 100 mm³, although for completeness, visualizations include all clusters regardless of cluster size. Those clusters which met the minimum size were subsequently mentioned and discussed.

The resulting ALE statistical maps were used in a conjunction analysis, in which each online and offline ALE maps were compared to identify regions that have significant overlapping activation/deactivation. Regions were identified with the Automatic Anatomical Labeling (AAL) template (Tzourio-Mazoyer et al., 2002) and MNI coordinates. Lastly, in order to investigate bias in activation patterns shown, a jackknife analysis was performed, whereby studies are systematically removed to determine how each cluster is impacted. This allows for an identification of clusters which are consistently activated across studies opposed to clusters which are dependent on a smaller subset of studies.

3.3 Results

3.3.1 Database search

The results of the NeuroQuery, NeuroSynth, and PubMed databases searches yielded 401, 116, and 1,260 studies respectively (1,777 total). Overall, 38 studies were retained. Of the accepted studies, 32 were categorized as online learning, and 6 as offline learning (Table 3.1).

Table 3.1 Studies included in the meta-analysis, with metadata and categorization.

Type	Study	N SS	N F	N RH	Age Range	Mean Age	Task Used
Online	Heun et al. (2004)	10	5	10	23-25	23.7	SRTT
	Oishi et al. (2005)	18	11	18	21-39	-	SRTT
	Penhune and Doyon (2005)	12	6	12	-	24.8	TMST
	Olson et al. (2006)	10	5	10	19-40	25	SRTT
	Macintosh et al. (2007)	12	7	12	-	29	Finger movement
	Anguera et al. (2007)	11	5	11	18-30	21.4	Spatial task
	Grafton et al. (2008)	10	-	-	-	-	Tracking task
	Tamás Kincses et al. (2008)	15	7	15	25-32	27.5	SRTT
	Rémy et al. (2008)	12	6	12	-	23.6	Coordination task
	Albouy et al. (2008)	90	-	90	19-28	-	SRTT
	Schlund et al. (2008)	10	-	10	18-50	-	Operant Conditioning task
	Fernández-Seara et al. (2009)	14	7	14	-	31	SRTT
	Orban et al. (2010)	32	17	32	-	23.9	SRTT
	Tomassini et al. (2011)	12	9	12	-	30.1	Tracking task
	Orban et al. (2011)	12	6	12	-	26.5	SRTT
	Pammi et al. (2012)	17	-	17	-	-	SRTT
	Watanabe et al. (2011)	15	0	15	19-47	22.8	Imitation task
	Lefebvre et al. (2012)	20	11	20	18-62	33.9	Cursor task
	Melcher et al. (2013)	33	18	33	-	24.7	Go/NoGO
	Lissek et al. (2013)	18	11	18	23-34	25.66	SRTT
	Cunningham et al. (2013)	24	20	24	-	25	Tracking task
	Tzvi et al. (2014)	17	10	-	20-32	-	SRTT
	Kornysheva and Diedrichsen (2014)	32	16	32	19-36	24.8	Motor sequence task
	Karim et al. (2017)	13	6	13	-	23.8	Motor sequence task
	Tracy et al. (2001)	5	3	5	21-27	23.6	Single finger opposition
	Drobyshevsky et al. (2006)	31	15	31	22-76	41	SRTT
	Sacco et al. (2009)	8	4	8	20.8-34.8	27	Finger movement
	Gheysen et al. (2010)	22	17	22	19-25	-	Serial color matching task
	Arima et al. (2011)	13	0	13	24-35	27.3	Tongue-training task
	Heitger et al. (2012)	34	19	34	20-30	23	Bimanual coordination training
	Müller et al. (2002)	7	0	7	23-46	-	SRTT
	Bo et al. (2011)	14	8	14	-	21.4	SRTT
	Gregory et al. (2014)	12	8	12	-	25	SRTT
Offline	Vahdat et al. (2011)	13	-	13	21-44	-	Reaching task
	Gryga et al. (2012)	15	5	15	22-32	-	Sequential pinch force task
	Tung et al. (2013)	24	15	24	-	30	SRTT
	Sidarta et al. (2016)	22	14	22	-	22.5	Reaching task
	Sami and Miall (2013)	24	-	24	-	23.6	SRTT

SS: Sample Size, F = Number of Females, RH = Number of right handed participants, SRTT = serial reaction time task (or alternative), TMST = temporal motor sequencing task.

3.3.2 ALE results

The ALE returned 20 significant ($p < 0.001$) clusters greater than 100 mm^3 in volume in the online dataset and 11 in the offline subgroup. Significant clusters, regardless of size, are shown for online motor learning (Figure 3.2A) and offline motor learning (Figure 3.2B). Cluster MNI coordinates, peak statistic, size, and associated region are presented in Table 3.2 and Table 3.3. The ALE analysis of online motor learning studies revealed significant activations of frontal, parietal, and temporal gyri, the cerebellum, putamen, pallidum, and thalamus, among other regions. The offline motor learning analysis revealed ALE clusters in the regions of the cerebellum, putamen, cingulum, pre and post-central gyri, frontal gyri, and supramarginal gyri.

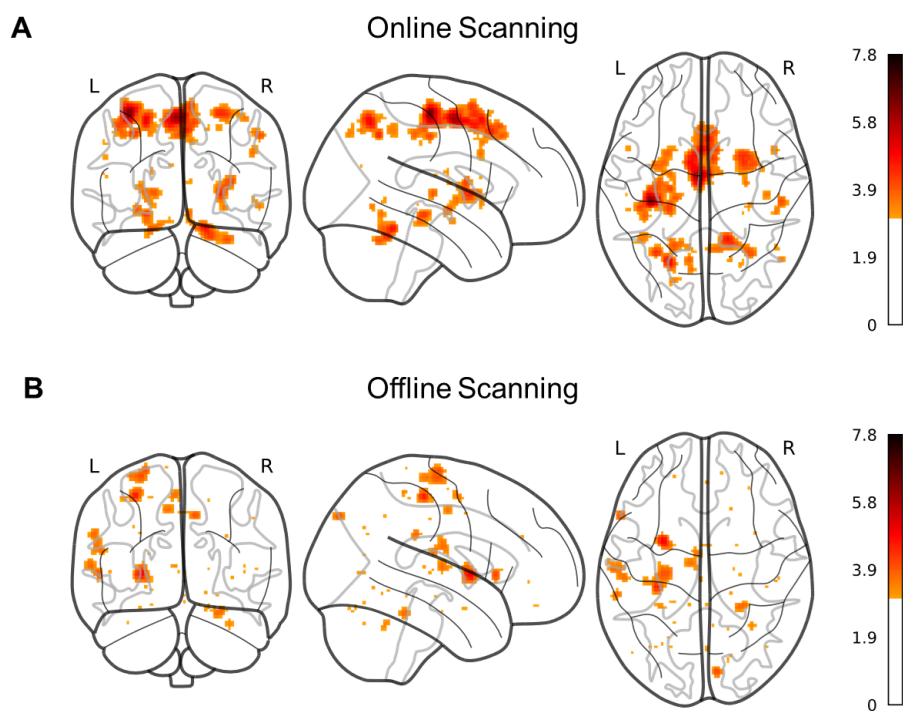


Figure 3.2 ALE results showing all significant clusters during A) Online and B) Offline motor learning. Colour bar represents magnitude of Z-statistic, and colouring threshold corresponds to $p < 0.001$.

Table 3.2 Significant clusters from ALE analysis of online motor learning.

Cluster ID	X	Y	Z	Peak Statistic	Cluster Size (mm ³)	Region (AAL)
1	-4	-8	54	6.177475	7960	L Supplementary Motor Area
2	-36	-24	58	6.420258	4216	L Precentral Gyrus
3	16	-50	-20	4.754861	2008	L Cerebellum, Lobule 4/5
4	-24	-64	52	5.034228	1544	L Superior Parietal Gyrus
5	26	2	2	4.502371	1528	R Putamen
6	26	6	56	4.476016	1400	R Superior Frontal Gyrus
7	-24	-28	-12	4.224487	680	L Parahippocampal Gyrus
8	-32	0	52	3.820151	584	L Middle Frontal Gyrus
9	-20	-54	-20	3.925206	536	L Cerebellum Lobule 6
10	52	-24	42	4.040562	440	L Postcentral Gyrus
11	-24	8	4	3.7921	408	L Putamen
12	-42	-50	46	3.475601	320	L Inferior Parietal Gyrus Gyrus
13	-18	-22	4	3.875925	264	L Thalamus
14	-30	-12	-8	3.958692	248	L Putamen*
15	-10	-64	56	3.813831	216	L Precuneus
16	42	-20	54	3.511122	160	R Postcentral Gyrus
17	-20	-78	44	3.437217	152	L Superior Occipital Gyrus
18	-22	-6	-2	3.602561	144	L Pallidum
19	48	10	30	3.596088	144	R Inferior Frontal Operculum
20	-28	-4	8	3.491422	136	L Putamen
21	-8	-70	46	3.597424	104	L Precuneus

*AAL: Automated Anatomical Labelling Atlas 3. *Indicates region closest to coordinates*

Table 3.3 Significant clusters from ALE analysis of offline motor learning.

Cluster ID	X	Y	Z	Peak Statistic	Cluster Size (mm ³)	Region (AAL)
1	-28	-20	68	4.004511	1128	L Precentral Gyrus
2	-28	2	2	4.944095	912	L Putamen
3	-32	-28	54	4.537863	592	L Postcentral Gyrus
4	-54	-22	18	3.648504	512	L Supramarginal Gyrus
5	8	-84	42	3.961662	288	R Cuneus
6	-60	-16	8	3.729101	264	L Superior Temporal Gyrus
7	24	-42	-24	3.703366	256	R Cerebellum
8	-8	-12	46	3.609021	232	L Middle Cingulum
9	-56	20	2	4.152583	224	Triangular Part of the Inferior Frontal Gyrus
10	-58	-34	30	3.702617	216	L Supramarginal Gyrus
11	-4	-6	56	3.588852	184	L Supplemental Motor Area

AAL: Automated Anatomical Labelling Atlas 3

3.3.3 Conjunction analysis

The results of the conjunction analysis revealed two clusters larger than 100 mm³ that had significant shared activation changes. The clusters are shown in Figure 3.3, with the coordinates, peak statistic, and cluster size. Cluster 1 corresponded to the left postcentral gyrus, and cluster 2 corresponded to L supplemental motor area.

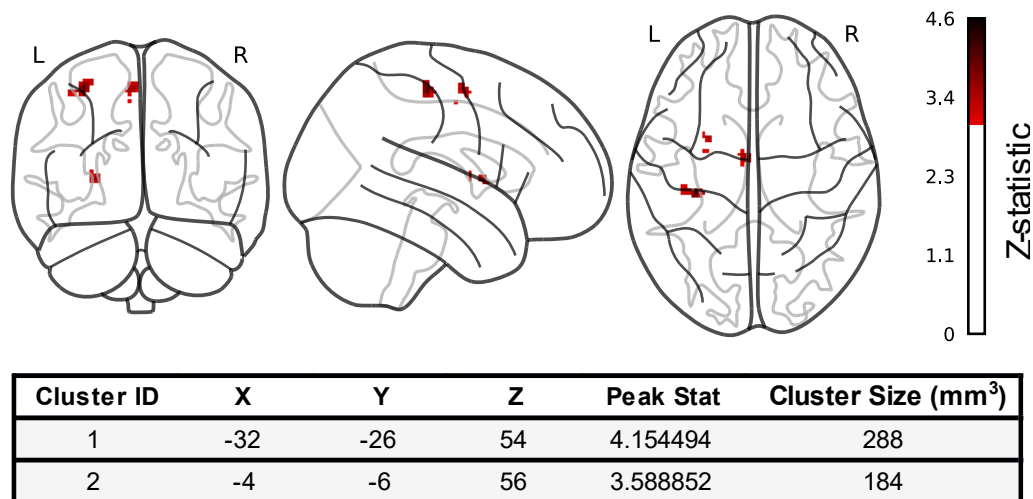


Figure 3.3 ALE results showing all significant shared clusters during both online and offline motor learning. Colour bar represents magnitude of Z-statistic (Peak Stat), and colouring threshold corresponds to $p < 0.001$. Peak State corresponding to Z-statistic is numerically represented with Cluster ID, MNI coordinates, and Cluster Size.

3.3.4 Evaluation of robustness

The results of the jackknife analysis showed that in each of the online and offline study groups, there was one cluster in which all studies included contributed activation. For the online group, a cluster with centre of mass at MNI coordinates [0, -20, 17] had contributions from all studies, corresponding to the midpoint between thalami. The offline group produced a cluster with centre of mass at MNI coordinates [-1, -23, 12] had contributions from all offline studies, likewise attributed to the midpoint between thalami. All cluster values are presented in the supplemental tables. Thus our results illustrate that both online and offline scanning studies implicated activation changes in (bilateral) thalami.

3.4 Discussion

The analysis found that both online and offline learning studies had consistent activations of several regions encompassing known circuits in motor acquisition and consolidation, corroborating current regional implication in both motor adaptation and its subsequent consolidation. Shared activation between stages was also confirmed in the motor and somatosensory cortices. This finding represents novel evidence of consistent motor networks activated across a variety of motor learning paradigms, and which are specific to either online and offline motor learning. Our findings of offline motor learning correlates also provide empirical support for the cortico-striatal network's involvement in motor learning, irrespective of the task (i.e., reaching, pinch force, SRTT), and the cerebellar-thalamic-cortical activation described during motor adaptive processes (Porrill & Dean, 2007).

3.4.1 Online motor learning

Findings from the analysis of studies that performed concurrent scanning and motor learning revealed that across both traditional serial reaction time task (SRTT) and visuomotor tasks, there was significant activation of previously described core regions associated with motor learning. Namely, in comparison to a recent quantitative meta-analysis of motor learning (Hardwick et al., 2013), we corroborate here that areas including the cerebellum, putamen, thalamus, precentral (M1) and parietal (SPL) regions are activated consistently during online motor learning experiments.

Placed in context of regional connectivity and motor learning circuitry, the regions implicated in our analyses are also supported by regions involved in motor system control, whereby M1 regions extend descending pathways to cerebellar regions and the basal ganglia, which in turn terminate on thalamic nuclei, creating a loop with output back to the M1 (Papale & Hooks, 2018). In the context of more recent framework, including that of adaptive motor control and learning, cerebellar involvement during online scanning would be consistent with the forward model of adaption, whereby consequences of motor commands are predicted and thus movement

is corrected (Shadmehr et al., 2010). Accordingly, this movement could then be corrected via the thalamus, which is a region also consistently activated online here, projecting to the cortices (i.e., motor) (Shadmehr & Krakauer, 2008). Furthermore, we note extensive activation of the subcomponents of the basal ganglia including putamen and globus pallidus, which too have been proposed to play a role in correction during controlled movement in the motor cortex (Magdoom et al., 2011). It would then appear that across the numerous task parameters and designs included here, consistent activation of regions involved in the adaptative pathway framework (e.g., cerebellum, thalamus, cortex, putamen) supports its role in motor learning, and identifies further numerous cortical regions likely involved in further processing, and which have previously been shown necessary for correct motor memory (Ebrahimi & Ostry, 2024).

It is noted that much of the activation presented in our findings appears left-lateralized, however we cannot draw conclusions about lateralization in motor learning, since we confirmed that 30 of the studies of online learning (93%) recruited participants that all boasted right-handed tendency (the remaining 2 studies did not clearly report handedness, and thus conclusions could not be drawn). Thus, it is likely that the pattern of lateralized activation is related to handedness (Andersen & Siebner, 2018).

3.4.2 Offline motor learning

The offline scanning analysis revealed that while regions such as the cerebellum, precentral gyrus, and putamen are activated in both the online and offline studies, there are several regions which were uniquely activated in the offline studies alone. The postcentral gyrus, supramarginal gyrus, cuneus, superior temporal gyrus, middle cingulum, inferior frontal gyrus, and supplemental motor area had clusters that were significantly activated during offline scanning. Interestingly, it has been shown in other work that regions such as the supramarginal gyri and postcentral gyri are indeed sensitive to training duration, and show activation changes during resting state scans following motor learning (Ma et al., 2011). The cuneus has also been implicated during resting state scans following motor learning, and has been suggested to be a part of a functional relationship with the sensorimotor areas (Roshchupkina et al., 2022), with

additional evidence showing that resting state cuneus functional activity correlates with motor learning (Mary et al., 2017). Pertaining to the offline regions identified here, the middle cingulum, putamen, post-central, pre-central, and cuneus have all been implicated as being a part of the cortico-striatal network (Debas et al., 2014). This network has been presented as a potential mechanism by which motor sequence consolidation occurs in the brain, as it has been shown that increased functional connectivity within the network relates to greater durability of motor trace through offline consolidation processes (Debas et al., 2014). We therefore find that many of the regions which are activated during resting state and post-motor learning scans were identified as regions of a network related to consolidatory processes in motor learning. Similarly to online motor learning, consolidation of motor memory in the context of adaptive motor learning framework would also explain cerebellar activation when arguably there is no movement to be corrected, and this dually implicates the consolidatory processes which may similarly be occurring during post-learning offline scanning. Animal studies (Attwell et al., 2002) have shown that the cerebellum is equally involved in retention, positing that adapted behaviour immediately following acquisition is dependent on cerebellar plasticity (Krakauer & Shadmehr, 2006).

It is important to note that offline scanning timepoints may not capture strictly ‘offline motor learning’ processes as with online motor learning. This is due to the fact that some tasks do not show offline improvements, such as the explicit SRTT (Tunovic et al., 2014). Despite this, we highlight that reported peaks are necessary for study inclusion, and thus by methodology, studies included here did report activational changes during the offline scanning point. Indeed the SRTT by Gregory et al. (2014) found correlations with next-day improvement, and that of Tung et al. (2013) investigated neural changes as a result of motor-task recency, and thus while the findings may not strictly represent offline motor learning processes, they do implicate regions association with the after-effects or changes to neural activation as a result of motor learning.

3.4.3 Shared activation between online and offline learning

The validation of both online and offline correlates of motor learning here, justify a further conjunction analysis in which we identify clusters which significantly overlap between

online and offline regional activation changes. There were two significant clusters (larger than 100mm³) that arose from the analysis, the first being the left postcentral gyrus, and the second being the left supplemental motor area. The implication of the left postcentral gyrus is consistent with motor learning both online and offline, given that it contains the primary somatosensory cortex (DiGuseppi & Tadi, 2023). Indeed the somatosensory cortex is a contributor to motor memory consolidation, as previous work has shown that theta-burst transcranial magnetic stimulation (cTBS) of the somatosensory cortex interfered with consolidation and eventual retrieval (Kumar et al., 2019). The role of the somatosensory cortex in online motor learning has also been extensively evidenced (Ito et al., 2016; Ostry et al., 2010; Wong et al., 2012), whereby the improvement trajectory of even implicit motor tasks can be altered with somatosensorial disruption (Vidoni et al., 2010). Similarly, the supplementary motor area (SMA) has also been shown have oscillatory changes reflecting performance improvement at post-training, and repetitive TMS (rTMS) of the SMA, directly after practice, reduced recall during a motor task (Tamaki et al., 2013; Tanaka et al., 2009). During online motor learning, the SMA is generally considered important for motor control (Nachev et al., 2007), and indeed this region was highlighted in the meta-analyses by Hardwick et al. (2013) and by Dahms et al. (2020). Thus, our findings suggest that while online and offline motor learning correlates have distinct activation which is consistent across numerous task types and populations, there is consistent activation of the SMA and somatosensory cortex across both groups. It should be noted, however, that despite having overlapping regional activation changes, the underlying processes which occur may differ dependent on the motor stage.

3.4.4 Consistency with adaptive online and offline motor learning framework

There is noted concordance between the results presented here, and the proposed adaptive motor learning theory put forth in recent years (Tani et al., 2014). Consistent online activation of the basal ganglia (notably putamen), cerebellum, motor cortices, SMA, and frontal regions can be placed easily within current theories of motor learning activation (Krakauer et al., 2019). This

goes beyond the traditional sequence learning, when considering that, despite a majority SRTT, the online motor learning studies included ranged beyond strict sequence learning, and so it is possible that activation of region, consistent with adaptive motor learning theories, is generalizable beyond sequence representation. Similarly, offline activation of regions including basal ganglia and cerebellar nuclei is consistent with error-based learning processes, whereby the cerebellum exerts normal inhibition of the motor cortices via the thalamus (Spampinato & Celnik, 2021). Consistent too, with offline activation patterns representing striatal-cortical network activation, is a noted shift from cerebellar to striatal-cortical network which is thought to represent improved forecasting models in the cerebellum associated with error correction and updating (Dahms et al., 2020). Overall then, our results support signatures consistent with the adaptive motor learning frameworks, and support consistent activation across numerous motor studies with varying tasks, durations, and specifications. We also report numerous instances of activation similarity between online and offline scanning, which suggests that regions associated with adaptive motor processes during learning are similarly engaged after online learning has ceased, but when continued learning processes still occur.

3.4.5 Limitations

Owing to a small number of studies that were eventually included in this analysis, we opted not to perform sub-analyses by the type of task used, as they would likely not provide generalizable results. Thus our findings should be taken in context of activation which is sufficiently represented across a variety of tasks (and indeed there were clusters which were activated across studies, regardless of task, see Supplemental Tables).

Regions between bilateral thalami likely did not survive the ALE or conjunction analyses since the individual activations were spread inconsistently across both thalami, and therefore producing no clear cluster reaching significance. As a result, the Jackknife analysis produced the midpoint of the cluster directly between thalami, suggesting thalamic involvement, but not with notable spatial concentration. Accordingly, the thalamus is also a region of strong consideration for implication in online and offline motor learning, but it is not extensively discussed here.

There is a substantial difference in the literature size sourced for each online and offline group. This is likely due to an (understandable) tendency to assess motor learning correlates concurrent with the task as opposed to independently, which greatly reduces the number of available fMRI studies that investigated consolidation or offline periods. Thus, while here we compare online and offline scanning point findings, there are likely more neural correlates of offline motor learning scanning which did not survive the analysis due to a small sample size, or inconsistent regional activation. Therefore, we highlight a clear imbalance between scanning which investigates online correlates of motor learning, and scanning during offline, post-learning periods.

We opted not to restrict study inclusion by a specified task, in order to obtain a generalized representation of regions implicated across varying motor-learning tasks. While this allows for a less-specific assumption about neural activation, it also prevents more specific assumptions about motor learning as it relates to characteristics such as finger vs arm use, explicit vs implicit knowledge, duration, and effort. These individual aspects almost certainly implicate different neural regions, and so individually (if sample size would allow) would produce different effects. We therefore stress that as a limitation to this work, our findings only narrate the regional implication to online and offline processes, highlighting their differences, but also echoing findings by those such as Tung et al. (2013), who also stress the importance of exercising caution when scanning after motor learning tasks, as placement time may prove critical to capture effects which may relate to offline processes or which may represent after-effects of recent motor learning.

3.5 Conclusion

Our findings identify unique and overlapping activation changes associated with online and offline motor learning correlates, supporting previous work and theories about regional contributions to motor learning. To our knowledge, this represents the first instance of a meta-analysis directly comparing online to offline motor learning, highlighting their respective overlapping and distinct signatures. While preliminary and restricted by study numbers, our

findings replicate previous meta-analyses which investigated online motor learning, and find support for the theory of the cortico-striatal network's involvement in motor consolidation and offline processes. By comparing online and offline ALE maps via conjunction analyses, we also concluded that both SMA and somatosensory regions have roles in online and offline motor learning. Overall, we also find evidence for adaptive motor learning processes persisting into offline scanning, likely reflecting consolidatory processes which represented a reactivation of the same adaptative pathways during online learning. Our findings support future work which seeks to investigate overarching processes that govern motor learning, and identifies targets for stimulation and modulation at either online or offline learning stages while supporting current models of motor learning. We also highlight the need for further research into offline motor learning correlates, given the lack of literature in this area reflected by small study availability.

Chapter 4 The Effect of Peripheral Nerve Stimulation on Motor Learning Behaviour

4.1 Introduction

There is a substantial history of modulating motor learning with alternating current stimulation, and successes have been promising but still considerably variable (Takeuchi & Izumi, 2021). Indeed recent mechanistic studies have further disrupted the field by revealing inconsistencies in the previously proposed theory behind its mechanism (Vöröslakos et al., 2018). As an alternative explanation, work investigating the peripheral effect of stimulation has surged, and a few promising studies combined with a longer history of animal work have created a strong forward step for modulation research in the motor field (Asamoah et al., 2019; van Boekholdt et al., 2021).

One of the most common methods of modulating motor learning is to pair stimulation of a brain region with task learning (e.g., the SRTT), and observe if differences in motor learning, perhaps via acquisition, occur as a result (Giustiniani et al., 2019a). One complicating feature to this approach is determining exactly *what* is being changed. Traditional motor learning studies using tDCS (in its transcranial form) would likely posit that the effects are driven by a rising of the resting potential in the targeted cortices, resulting in enhanced neuroplasticity and thus better learning (Chan et al., 2021), an experiment which indeed has successfully been carried out (Mosayebi Samani et al., 2019). However, the enhancement of motor learning does not need to be restricted to the motor cortex. The adaptive motor learning framework which approaches motor learning in the context of (Bayesian-style) predictive coding, considers the continued adaptation and adjustment of motor action via the cerebellum and thalamus as the basis for continued learning which later consolidates (all as a function of regional plasticity), and exposes as behaviour (Huang et al., 2011; Krakauer, 2009; Krakauer et al., 2019). In this definition, there is much more opportunity to modulating motor learning than simply stimulating the motor cortex, and although success in tDCS over the M1 should not be ignored, the variability of results and

methodological constraints posit the need for alternative research (Bland, 2021; Vöröslakos et al., 2018).

Following the current motor learning framework, we consider that the continued adaptation and utility of an internal model of motor action contributes to motor learning, and it has accordingly been theorized that the LC integrates prediction errors to compute model failures, which is supported with learning rate alteration as a result of LC stimulation in rodents (Glennon et al., 2019). This model then assumes that LC output is highest when internal models are inaccurate, with output signalling either the statistical quantity of uncertainty (Angela & Dayan, 2005), or the error itself (Sales et al., 2019). We can therefore leverage this model when we consider that NITESGON has been shown to modulate LC activity when paired with a task (Vanneste et al., 2020). LC stimulation has also recently been proven to induce task-relevant motor plasticity in rodents, in a study where stimulation at three frequencies (3 Hz, 10 Hz, or 30 Hz) during motor training found that 10 Hz stimulation, but neither 3 Hz nor 30 Hz, increased motor map representation of task relevant musculature (Tseng et al., 2024). This essentially represents an upstream confirmation of previous studies by showing that VNS achieves a similar effect, dependent on NE and ACh (Daniel R. Hulsey et al., 2016; Hulsey et al., 2019). Frequency-specific effects of motor learning are commonplace in tACS-motor research (Bologna et al., 2019; Wach et al., 2013), and are likely attributable to endogenous neural oscillations, and the effect of the induced stimulating frequency. These are also true for the LC, wherein LC neurons exhibit a phasic form of firing characterized in the 10-20 Hz range (Devilbiss & Waterhouse, 2011) (a possible explanation for the frequency-specific modulation observed in Tseng et al. (2024)). Accordingly, we use different stimulating frequencies in this study to determine if frequency-specific effects are reproducible in human peripheral nerve stimulation. Our choices included a sham stimulation condition, and 1 Hz stimulating control (Luckey, McLeod, Mohan, & Vanneste, 2022; Vanneste et al., 2020), a 20 Hz stimulation condition matching phasic firing range of the LC neurons and endogenous motor beta oscillations (Barone & Rossiter, 2021; Kilavik et al., 2013; Krause et al., 2016; Miles Wischniewski et al., 2019), and a 40 Hz stimulation

condition consistent with low gamma range (also shown to modulate motor learning behaviour in previous studies, but above LC phasic firing range) (Giustiniani et al., 2019a; Giustiniani et al., 2021; Miyaguchi et al., 2022).

In human research, motor learning can be approximated via behavioural data such as skill (a performance difference between a learnable motor sequence and random one) (Diedrichsen & Kornysheva, 2015; Kornysheva & Diedrichsen, 2014). In the conventional SRTT, the participant uses their fingers mapped to a visual cue, and repeats button pressing in response to the cue over a series of trials (Schwarb & Schumacher, 2012). The cues are either sequential (a repeating pattern of n items), or random. The repeated and therefore *learnable* sequence is thus acquired during the course of the experiment, while the random unlearnable cues represent an interference resulting in performance errors and slowing when conditions are interleaved (Robertson, 2007). Through this process under the adaptive motor learning framework, the brain would (during sequence blocks) rely on a model whereby the next response is predicted (and errors integrated). Dependent upon the correctness of this prediction, this model would be updated accordingly (Huang et al., 2011). Such process describes procedural learning and forms the basis for the measurement of motor learning. The SRTT also has several ‘customizable’ parameters that warrant consideration including the implicitness of the sequence, the length of the sequence, the duration of the task, and the strength of stimulation. Here we pursue a dual-task paradigm in order to enhance difficulty and to encourage sequence-blinding (although this is not without drawbacks, see Röttger et al. (2021)). Participants were asked to return after 24 hours and repeat a variation of the task in order to gauge the effect of the stimulation on the consolidation or retention of this learning (Gastrock et al., 2024).

Our main goal is to determine the effect of peripheral nerve stimulation on motor learning, as measured by behavioural performance on a serial-reaction time task (SRTT). To do so, we leverage several features of the motor learning task including handedness, goal awareness, inter-manual transfer, and consolidation periods to better understand the effect of NITESGON on motor learning and retention. We propose here that NITESGON, as has been shown previously

in memory, would activate the occipital nerve resulting in altered LC firing via afferent connection to the brainstem (Vanneste et al., 2020). Altered LC activity could, as has been shown in rodents, modulate motor learning behaviour by changing neural gain (which has also been shown in rodents), and in a sense enhance the adaptive and predictive weighting during the learning process (Vazey et al., 2018) (see also a thorough review in Jordan (2024)). Furthermore, and following pre-translational work, activation of the LC via peripheral stimulation could also modulate plasticity via NE or ACh-driven plastic changes in the engaged region (Hays et al., 2013; Daniel R. Hulsey et al., 2016; Rasmusson, 2000).

As an additional experiment, and following previous work (Vanneste et al., 2020), we will also be using a topical anesthetic to block the occipital nerve site of stimulation while delivering NITESGON at the determined optimal frequency. This comparison will allow us to observe if there is a reduction in effect with nerve blocking and quantify the contribution of transcutaneous and transcranial mechanisms. We expect based on previous work that pairing NITESGON and a motor task should alter motor learning behaviour, and further that this modulation should be frequency specific. We also expect that after identifying the optimal stimulating frequency, nerve blocking should reduce its effects, indicating a peripheral mechanism.

4.2 Methods

4.2.1 Participants

Sixty participants were originally recruited to participate in the study. Participants were required not to have any tES contraindications, and no neurological or psychiatric diagnoses. The beck depression inventory (BDI-II) and beck anxiety inventory (BAI) were used to exclude individuals above a moderate/severe category of depression (20+) or anxiety (16+). Handedness was assessed using a short form version of the Edinburgh Handedness Inventory (EHI). Informed written consent was given prior to participation. Participants were randomly assigned to either sham, 1 Hz, 20 Hz or 40 Hz stimulation groups. The sham condition had $n = 15$, mean age = 24.0 years, $SD = 6.5$ years (12F, 3M). The 1 Hz had one subject removed because they did not return

on day 2, ($n = 14$), mean age = 21.1 years, SD = 3.8 years (13F, 1M). The 20 Hz condition had 1 subject removed due to awareness of the sequence, and 1 subject removed due to BDI/BAI exclusion ($n = 13$) mean age = 25.3 years, SD = 3.6 years (8F, 4M). The 40 Hz condition had $n = 15$, mean age = 21.1 years, SD = 3.03 years (11F, 4M).

In a secondary experiment, an additional 15 participants were recruited into a 20 Hz stimulation group which also received a topical anaesthetic. One participant was removed due to exclusion criteria for medication. Participants received additional screening for Lidocaine/Prilocaine exclusion. Following collection, one participant was additionally removed because they took longer on the task than upper limit of the stimulation delivery (20+ minutes). Thus the 20 Hz + lidocaine group ($n = 13$) had a mean age = 26.1 years, SD = 3.1 years (8F, 5M). The sample size, age, handedness, gender, BDI and BAI scores for each group are presented in Appendix II for completeness.

4.2.2 Experimental procedure

Participants were asked to complete a version of the serial reaction time task (SRTT) while receiving stimulation and return for a second session on the following day. Participants were not informed of which group they were assigned but were told that there were both active and control conditions, and both produce similar sensations due to the blinding procedure. Following the 15-minute SRTT, participants were asked to return 24 hours later to complete a shortened version of the task. Participants used their non-dominant hand during the training (day 1) session. The hand was chosen based on the EHI, and if the participant was classified as mixed handed, the hand not used for writing was chosen as non-dominant.

The training day 1 SRTT followed a typical motor learning design and included the presentation of a visual stimulus in the form of 4 vertical rectangles (Figure 4.1) on a 60cm × 34cm monitor with 75 Hz refresh rate and 1024×768 resolution. Participants sat approximately 60 cm from the screen and placed their 2nd through 5th digits (pointer finger to little finger) on the H, J, K, and L keys on a keyboard. (If non-dominant was left, little finger was on H, and if non-dominant was right, pointer finger was on H). Participants were asked to press the button on the

keyboard corresponding to the location of the visual stimulus (rectangle darkened). Participants were not told of the existence of any sequence and were simply instructed to respond as quickly and accurately as possible, with no preference for either.

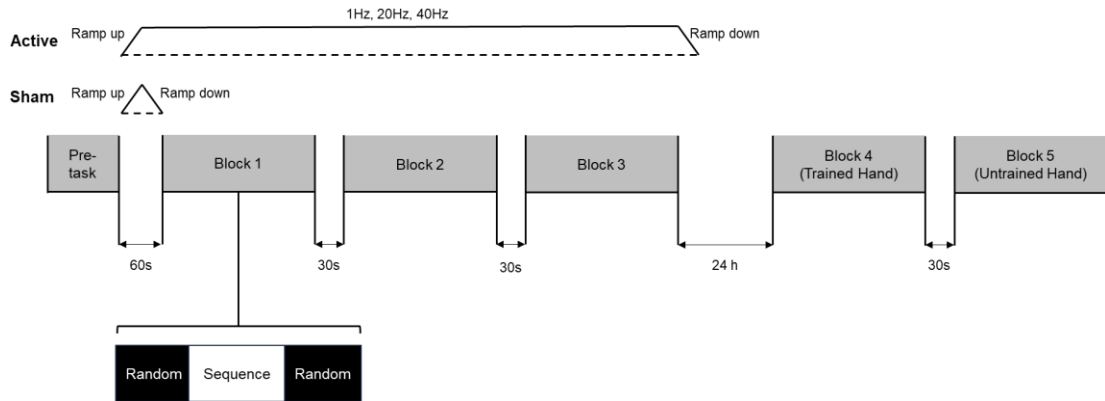


Figure 4.1 Task schematic and design. Day 1 and day 2 task design indicating duration of active and sham stimulation. Arrows indicate breaks with respective durations, and random and sequence sub-blocks indicate non-repeating or repeating trial structures respectively.

A pre-task block of 25 random trials was used to introduce the participants to the task and confirm understanding. The task design included sequence blocks (S) and random blocks (R) in the following pattern: R-S-R-break-R-S-R-break-R-S-R. All random blocks were 50 trials in length, and the three sequence blocks were 120, 200, and 120 trials in length, respectively. Breaks were self-paced, but with a cue to begin after 30 seconds to indicate when participants could continue. Sequence blocks consisted of a repeating 8-digit pattern (2nd order). While participants were performing the task, they were also asked to keep a running count of an auditory stimulus. This was included to prevent conscious awareness of the sequence, and to increase difficulty of the task in general in order to prevent ceiling effects. At each trial, a sound was presented with either a low (400 Hz) or high tone (2000 Hz) lasting 400ms (or until button press). Participants were asked during the warm-up block to indicate if they could hear a difference between the tones (to exclude those unable to distinguish between them), and to ensure task compliance and understanding. Participants were instructed to keep a running count of the higher tones, while ignoring the low tones. Tones were presented with 50% probability to avoid an oddball effect. At

the end of each block during the break periods, participants were asked how many tones they counted, and then were instructed to begin counting again from zero. Following the task completion, participants completed a tES exit-questionnaire probing sensations felt during stimulation (Appendix II) and provided a guess of which stimulation group (active or control) they were assigned.

After 24 hours, participants returned to perform a shortened version of the task without stimulation and without any auditory stimulus to better gauge the extent of motor learning without a secondary task. Participants first completed one grouping of R-S-R on their non-dominant hand, and after a 30 second break, were asked to complete the remaining blocks on their dominant hand. The sequence of button presses was inverted for the dominant hand, such that the sequence was now the mirror-version of the non-dominant hand sequence (consistent with a goal-directed action mode, as opposed to stimulus-driven action (Cohen et al., 2005)). During both training day 1 and testing day 2 sessions, the task followed the same design, which included presentation of a visual stimulus with auditory stimulus of the same length, which when the button was pressed (correct or incorrect) would disappear for 400ms, and the next stimulus would be presented (Figure 4.2).

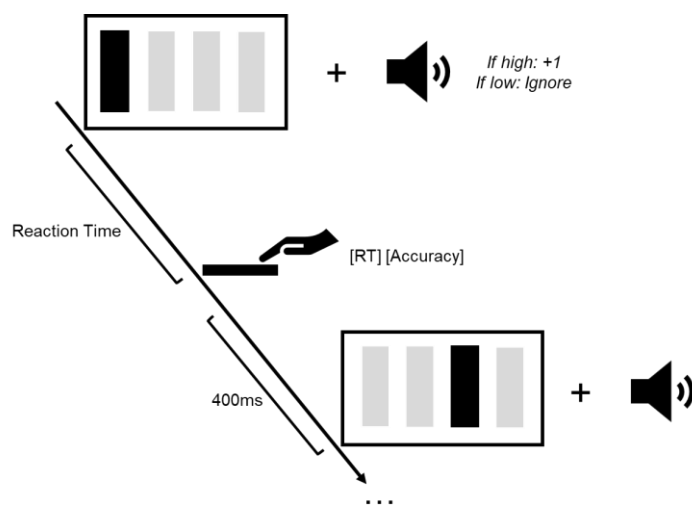


Figure 4.2 Trial structure design. Trial design including visual stimulus, reaction time duration, and inter-trial interval.

4.2.3 Stimulation protocol

Stimulation was delivered to the C2 dermatome using a DC-Stimulation Plus device (NeuroConn, Germany). Saline-soaked electrodes (35cm²) were placed with anode on the left side of the dermatome, and cathode on the right, wires facing inward (Figure 4.3). This placement follows previous work which identified the occipital nerve as a target (Luckey, McLeod, Huang, et al., 2022; Luckey, McLeod, Mohan, & Vanneste, 2022; Vanneste et al., 2020). During the sham stimulation condition, stimulation delivered in a sinusoidal wave was ramped up over 30 seconds to 1.5mA at 1 Hz frequency, which lasted 60 seconds, followed by a 30 second ramp-down, before the participant was told to begin the task. For the active conditions (1 Hz, 20 Hz, 40 Hz), stimulation was delivered for the duration of the training session (~15 minutes, 5 mins $\times$ 3 blocks) at 1.5mA at the given frequency, following a ramp-up and ramp-down of 30 seconds (1 Hz) or 2 seconds (20 Hz, 40 Hz). Participants were instructed to begin after 30 seconds to allow time for complete ramp-up.

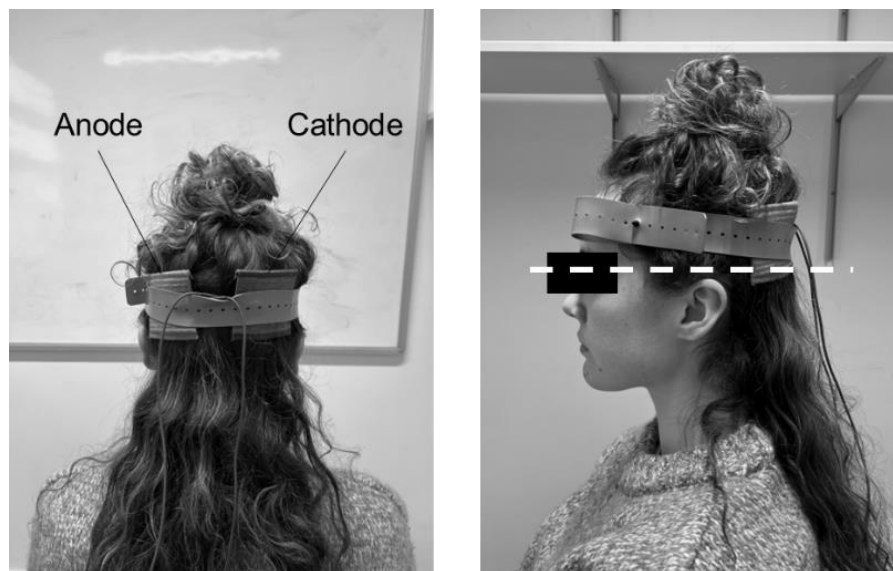


Figure 4.3 Electrode placement. Anode and cathode of electrode leads are placed on the left and right C2 dermatome respectively. Placement landmarked to top or ear, with cables facing inward.

Frequency conditions were selected based on previous work and theoretical applicability to motor learning. 20 Hz was selected since previous research has shown that 20 Hz tACS contributes to motor learning compared to sham or 35 Hz stimulation, and specifically yielded better robustness against interference (Krause et al., 2016; Pollok et al., 2015b). Further, increased motor cortex plasticity following 20 Hz tACS follows the same goal as previous studies investigating vagus nerve stimulation for motor plasticity, and thus 20 Hz stimulation of the occipital nerve may similarly improve motor learning via inducing plasticity in humans (Daniel R. Hulse et al., 2016; Hulse et al., 2019; M. Wischniewski et al., 2019). In contrast, 40 Hz (low gamma) has been shown in previous work (Luckey, McLeod, Mohan, & Vanneste, 2022) to improve word acquisition in an associative memory recall task, suggested to be due to enhanced attention. In the motor domain, gamma stimulation has also been shown to induce changes in inhibition, relating to ability on a motor task, suggesting that tACS might alter motor learning performance via a GABAergic mechanism (Nowak et al., 2017; Nowak et al., 2018), and improve motor learning (Giustiniani et al., 2019a). Thus, we chose to pursue 20 Hz and 40 Hz as active stimulation conditions to determine if either frequency would influence motor learning via a peripheral nerve. A 1 Hz stimulation control (Voskuhl et al., 2016) was chosen as it falls below physiologically significant oscillations for motor learning, and has been shown to previously effective in acting as a blinding condition which matches the continuous sensation of an active condition, but without inducing noticeable behavioural effects (Giustiniani et al., 2019a; Luckey, McLeod, Mohan, & Vanneste, 2022). Thus, the sham group and 1 Hz group both function as control conditions, and 20 Hz and 40 Hz groups function as active conditions.

4.2.4 Topical anaesthetic

In a secondary experiment, we pursued an addition group which received 20 Hz stimulation with topical anaesthetic to block the proposed nerve pathway, as has been shown previously (Asamoah et al., 2019; Vanneste et al., 2020). Lidocaine/prilocaine anaesthetic cream acts by blocking sodium channels, increasing the threshold for electrical stimulation to elicit an effect and preventing excitatory transmission (Hermanns et al., 2019). Accordingly, if the

proposed stimulation mechanism is mainly peripheral, the behaviour effects elicited should be reduced or removed by increased the stimulation threshold and/or preventing propagation along the occipital nerve. Conversely, if the effects are driven by a traditional transcranial mechanism, we propose that no difference in modulation between 20 Hz stimulation and 20 Hz stimulation + lidocaine groups should occur, since the activity of peripheral nerves would not influence the field distribution in the skull and subsequently neural tissue. EMLA 5% cream (25mg lidocaine, 25mg prilocaine) was administered in a rectangular area underlying both electrodes and the space between them 30 minutes prior to stimulation. Participants completed the task as normal and were not explicitly told the cream was to alter a modulation effect, but rather that the cream was used to reduce discomfort in order to prevent placebo-based effects.

4.2.5 Analysis

All data were analyzed in Jasp (JASP, 2024). To determine if there were differences between the groups in terms of reported group-guess or sensations, an analysis of variance (ANOVA) for each type of sensation was conducted. A Chi-Squared test was performed to determine if there was a significant effect of group condition on group guess. Counts of tones across the three training blocks were compared between groups using a repeated measures analysis of variance (rmANOVA), with block as the repeated measure, and group as the factor.

Behavioural data was preprocessed for each individual offline. Reaction times were removed if they were $< 200\text{ms}$ (Röttger et al., 2021) or exceeded three standard deviations above the mean. The mean reaction time for each trial type was calculated including reaction times during incorrect button presses. Accuracy was calculated as the proportion of correct responses out of the total number of responses. Skill in this chapter refers to a measure which compares the reaction time during sequence blocks to the subsequent random blocks. Skill was calculated by taking the mean reaction time of the last 50 sequence trials, and the mean reaction time of the first 50 random trials, subtracting the values to produce a difference in reaction time between sequence and random reaction times, and dividing the difference by the random reaction time to create a relative measure of difference (Equation 4.1). This is designed to account for existing

difference in random response times in individuals, creating a more normalized and comparable measure (Meissner et al., 2018).

Equation 4.1

$$Skill = \frac{Mean\ RT_{Random(first\ 50)} - Mean\ RT_{Sequence(last\ 50)}}{Mean\ RT_{Sequence(last\ 50)}}$$

Analysis of reaction time and accuracy were carried out using a generalized linear mixed model (GLMM) where block and group were included as factors, and reaction time/accuracy as the outcome measure. A GLMM was used to analyze the main effect of group on each relative block improvement, and deviation contrasts were performed to analyze each group compared to the others. In order to determine the relationship between skills levels, for example to determine if late-train skill correlated with skill levels 24 hours later, Pearson correlations were performed between early and late-stage skill, late-stage and untrained skill, and late-stage and trained skill.

Hand transfer index was calculated using Equation 4.2 in order to determine the change in skill when participants changed their hand from the trained to untrained hand on day 2. Difference in hand transfer index between groups was analysed using a GLMM.

Equation 4.2

$$Hand\ Transfer\ Index = \frac{Skill_{Trained} - Skill_{Untrained}}{Skill_{Trained} + Skill_{Untrained}}$$

The above statistical approaches were carried out in the secondary experiment which only compared 20 Hz stimulation with and without the Lidocaine application, and these data are presented separately in 4.3.6.

4.3 Results

4.3.1 No sensory or compliance differences across stimulation conditions

There were no differences between groups in any of the sensations reported (Table 4.1), suggesting against a placebo-driven effect based on sensation. The results are also presented visually in Appendix II. There were no significant differences between groups in the number of counted tones across the three blocks ($F(3,51) = 0.58, p = 0.627$), suggesting against an effect of counting-compliance on the task performance, in which less counting could allow for more attention directed to the task (Figure 4.4). The proportion of participants guessing the active/non-active condition did not differ by group $X^2(3, N = 58) = 6.32, p = 0.09$ (Figure 4.4). Therefore, there is no significant indication that participants were aware of their condition allocation based on guess or reported sensations. All participants reported normal (subjective) sleep duration between the training and testing days.

Table 4.1 Mean sensation intensity for each group following stimulation with ANOVA results.

Sensation	Mean sensation $\pm$ SD ^a				ANOVA (3, 54)	
	Sham	1 Hz	20 Hz	40 Hz	<i>F</i>	<i>p_{uncorr}</i>
Headache	1.06 $\pm$ 0.26	1.14 $\pm$ 0.36	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.34	.27
Neck Pain	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.14 $\pm$ 0	1.00 $\pm$ 0.00	1.05	.38
Scalp Pain	1.13 $\pm$ 0.52	1.13 $\pm$ 0.52	1.07 $\pm$ 0.27	1.13 $\pm$ 0.35	1.36	.94
Tingling	1.27 $\pm$ 0.46	1.57 $\pm$ 0.51	1.43 $\pm$ 0.36	1.73 $\pm$ 0.59	2.17	.10
Itching	1.06 $\pm$ 0.26	1.14 $\pm$ 0.36	1.28 $\pm$ 0.61	1.33 $\pm$ 0.61	0.95	.42
Burning	1.06 $\pm$ 0.26	1.14 $\pm$ 0.36	1.07 $\pm$ 0.28	1.20 $\pm$ 0.41	0.50	.68
Skin Redness	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	-	-
Sleepiness	1.07 $\pm$ 0.26	1.36 $\pm$ 0.63	1.21 $\pm$ 0.43	1.47 $\pm$ 0.83	1.33	.27
Trouble Concentrating	1.40 $\pm$ 0.50	1.50 $\pm$ 0.65	1.93 $\pm$ 1.27	1.67 $\pm$ 0.82	1.05	.38
Mood Changes	1.20 $\pm$ 0.41	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.13 $\pm$ 0.52	1.28	.29

^a1 = absent, 2 = mild, 3 = moderate, 4 = severe.

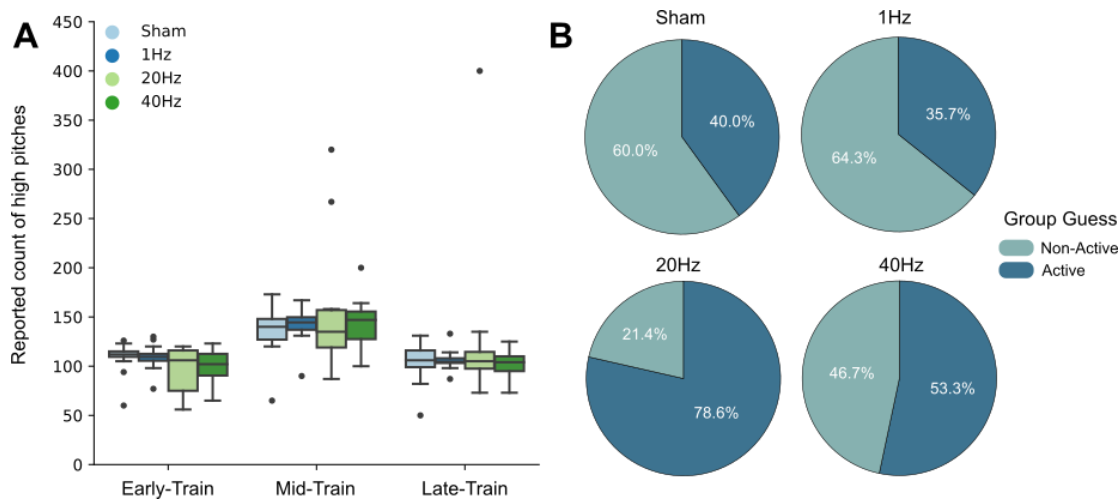


Figure 4.4 Pitch counting by group and group-guess. A) Group-counts by training block. B) Group-guess represented by group allocation. Non-active guess indicates belief of placement into sham/control group, active group indicates belief of placement into stimulation group.

4.3.2 20 Hz stimulation modulates motor acquisition

On day 1, the GLMM with factors for block, group, and their interaction revealed a significant effect of group ($F(3,477) = 16.249, p < 0.001$) and block ($F(8,477) = 8.018, p < 0.001$). There was no significant interaction ($p = 0.995$). Subsequent deviation analyses found that the 1 Hz and 40 Hz groups expressed larger response times, with an estimated increased of 0.16 and 0.025 s respectively (1 Hz: $t(477) = 2.316, p_{\text{adj}} = 0.021$; 40 Hz: $t(477) = 3.560, p_{\text{adj}} < 0.001$). The 20 Hz group instead showed a significant decrease in response time, with an estimated deviation of 0.049 s, $t(477) = -6.831, p_{\text{adj}} < 0.0001$) (Figure 4.5).

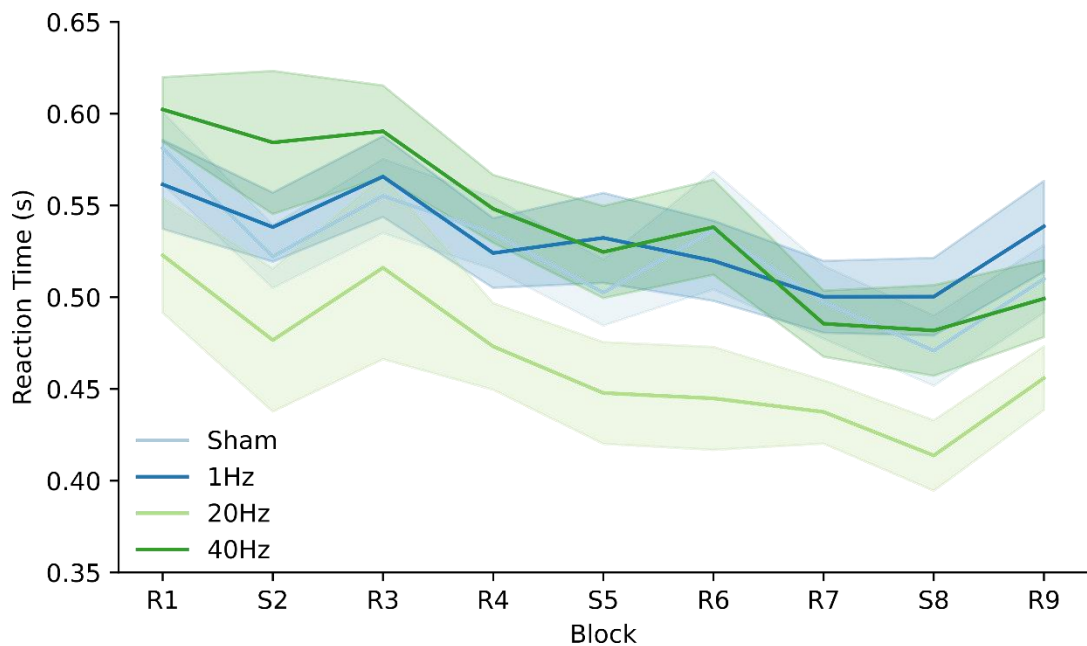


Figure 4.5 Mean reaction time during day 1 training and group. Colour indicates group identity. R blocks indicate random trial blocks, and S blocks indicate sequence trial blocks. Shaded region indicates SEM. * $p < 0.05$.

We consider the skill metric as a stable measurement of learning, as opposed to response time and accuracy which do not specifically capture sequence-specific learning. A GLMM designed for the interaction between group and blocks found no significant effect of group ($p = 0.241$) but a significant interaction between group and block ($p = 0.005$). A deviation analysis was thus carried out to understand if any group differed throughout training and found a significant negative deviation in the 1 Hz and 20 Hz group during block 2 skill ($p_{\text{adj}} = 0.002$ for both). In the final block, however, only the 20 Hz group showed significant positive deviation from mean skill ($p_{\text{adj}} = 0.011$) (Figure 4.6).

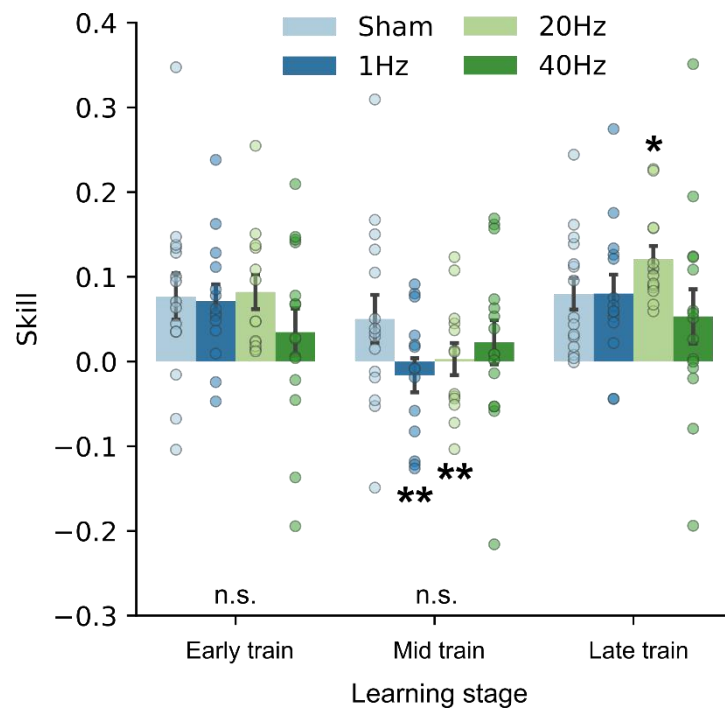


Figure 4.6 Skill measures during day 1 training. A) Early, Mid, and Late-Train skills across groups. $*p < 0.05$, $**p < 0.01$.

4.3.3 No difference in sequence retention or hand transfer

The same GLMM for response time was carried out on day 2 but with an additional effect of Trained or Untrained hand. Response time was significantly affected by the block ($p < 0.001$) and group ($p = 0.028$). While no group significantly differed from the 20 Hz group, it was found that there was a significant difference overall between the sequence subblock and subsequent random interference block ($p < 0.0001$, estimated difference: 0.034 ± 0.008 s) (Fig. 4.7A). We concluded that the differences in response time produced by 20 Hz stimulation were obvious during learning, but did not persist 24 hours later, although this could be due to overnight consolidation difference between groups or a ‘ceiling effect’, and across all groups there was still evidence of learning 24 hours later. The same GLMM on day 2 test skills found a significant effect of hand ($p < 0.001$), but not of group or interaction between them. Skill was greater on the trained hand compared to the untrained hand ($p_{\text{adj}} < 0.0001$, estimated difference: 0.077 ± 0.017 s) (Fig. 4.7B).

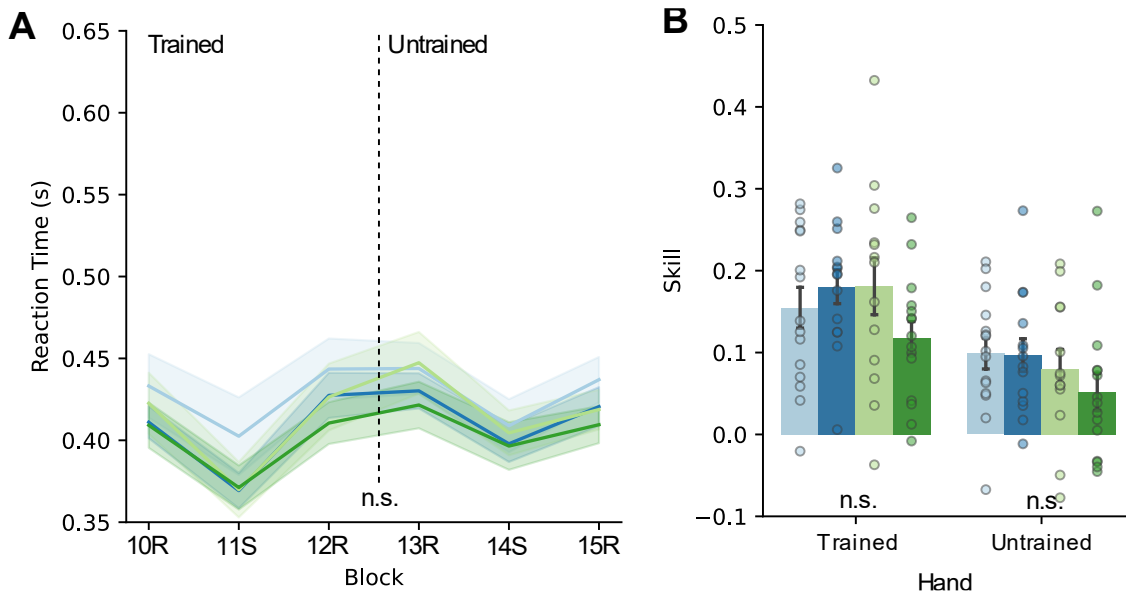


Figure 4.7 Day 2 testing reaction times, skill and skill transfer. A) Mean reaction time of groups across trained and untrained hands during day 2 testing. R and S denote random and sequence blocks respectively. B) Skill (ms) on trained and untrained hand during day 2 testing. Error bars indicate SEM.

4.3.4 Accuracy could explain differences in skill acquisition

We wanted to address if the difference in response time during training could be attributable to altered task adherence such as compensated accuracy, and so we pursued an analysis of the training day accuracy. A GLMM revealed a significant effect of group ($p < 0.001$) and block ($p < 0.001$). The 20 Hz group had a significantly lower accuracy relative to other groups (all $p_{\text{adj}} < 0.001$). Thus, the effect of response time improvement was also characterized with reductions in accuracy of between 1.5-2.5% (Figure 4.8).

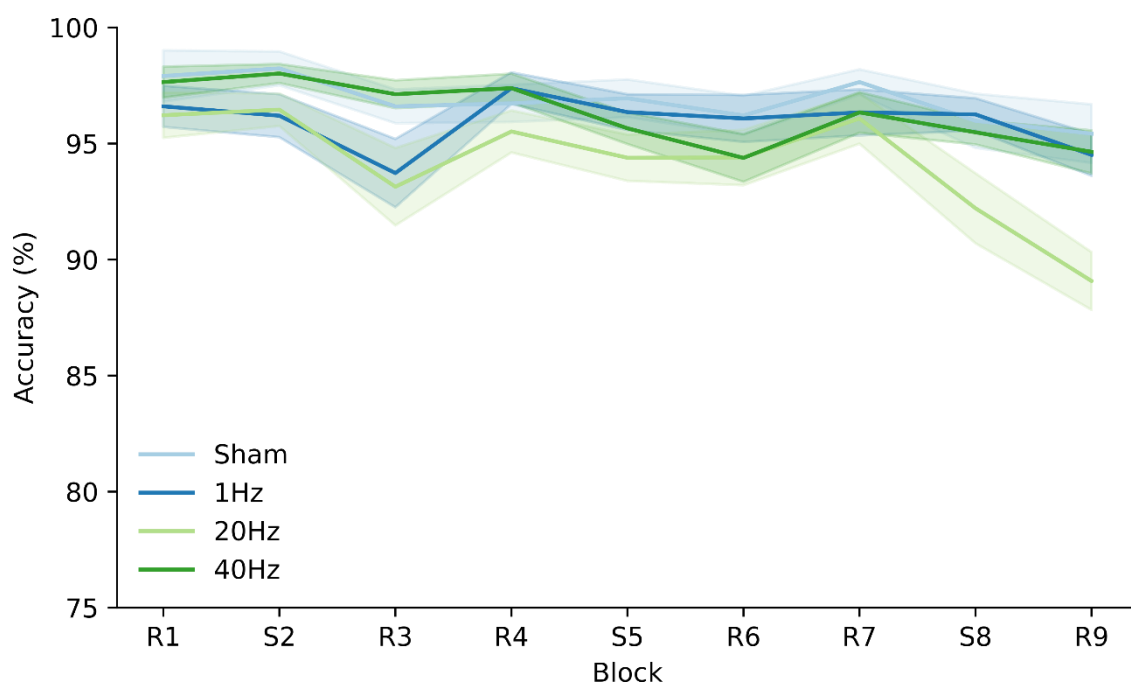


Figure 4.8 Mean accuracy by block during day 1 training. Accuracy (% correct responses) by block for each group during training. R and S indicate random and sequence blocks respectively. Shaded area indicates SEM. $*p < 0.05$.

4.3.5 Late-skill predicts trained and untrained testing skill 24 hours later

We analyzed the relationship between early-train (block 1) and late-train (block 3) skill on day 1 in order to determine if existing motor skill at the start of learning predicted motor skill following learning. A Pearson correlation did not find a significant relationship between early-train and late-train skill across all groups ($r = 0.12$, $p = .36$) (Figure 4.9A). There was also no relationship between early-train skill and skill on day 2 on the trained ($r = 0.25$, $p = .06$) (Figure 4.9B) nor untrained hand ($r = 0.08$, $p = .54$) (Figure 4.9C). Thus, it does not appear that existing motor skill on the task at the early-train block influenced the subsequent learned skill, nor retention or hand transfer. We can then more confidently claim that differences arising are more likely attributable to the intervention and learning blocks.

There was a significant correlation however, between late-train skill and day 2 trained hand skill ($r = 0.34$, $p = 0.01$) (Figure 4.9D) and untrained hand skill ($r = 0.35$, $p = .008$) (Figure

4.9E). Furthermore, there was a significant correlation between the trained and untrained hand skill on day 2 ($r = 0.30, p = .02$) (Figure 4.9F).

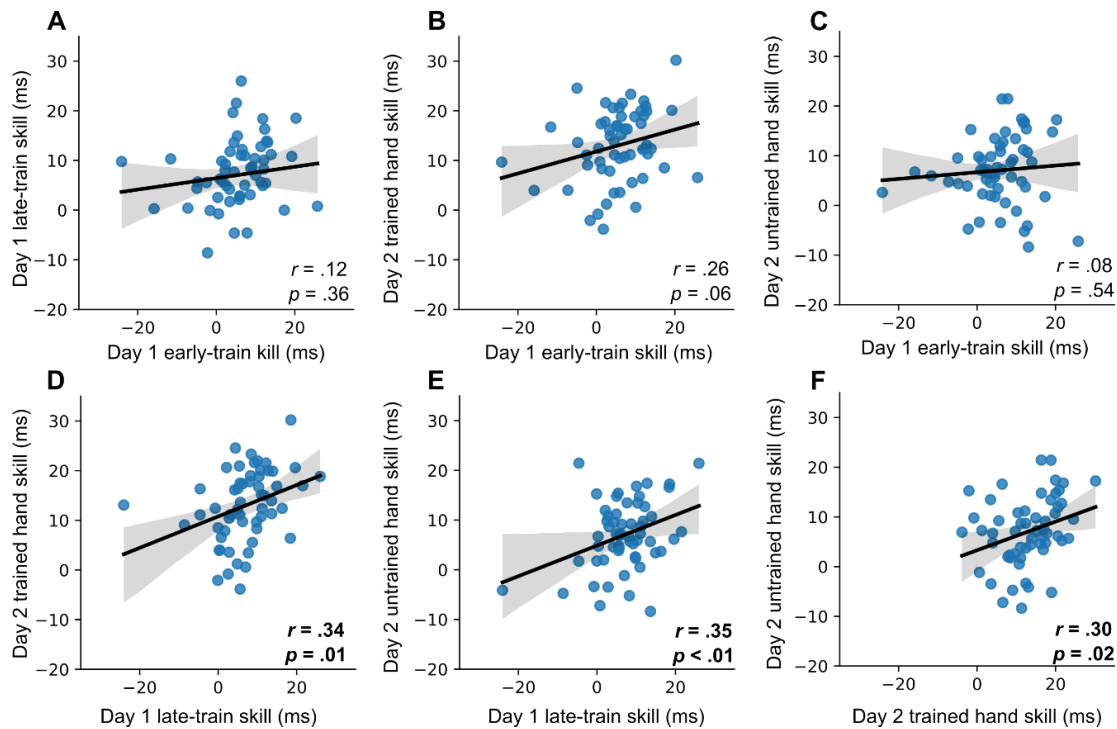


Figure 4.9 Correlation between skills across training and testing. Correlation between motor skill measures across all groups for A) early and late-training skill on day 1, B) early-training skill and day 2 trained hand skill, C) early-training skill and day 2 untrained hand skill, D) late-train skill and day 2 trained hand skill, E) late-train skill and day 2 untrained hand skill, and F) day 2 trained hand and day 2 untrained hand skill. Pearson's correlation coefficient and p value are presented with visualization, shaded area indicates 95% confidence interval of regression line. Bolded statistics are significant.

4.3.6 Topical anaesthetic reduces 20 Hz behavioural improvement

We first investigated if there were sensation differences between the 20 Hz and 20 Hz + lidocaine group. Independent sample t-tests were performed across the 10 sensation measures reported following stimulation. 6 of the reported measures produced no measurable difference (all groups reported mild), and there was no significant difference between groups in the remaining 4 measures; tingling ($t = 1.19, p = .243$); itching ($t = -0.079, p = .935$); sleepiness ($t = -0.176, p = .862$); and trouble concentrating ($t = 1.140, p = .266$). There was however a significant difference in group guess (20 Hz: 10 active guess, 3 nonactive guess, 20 Hz + lidocaine: 3 active guess, 10 nonactive guess) $X^2(1, N = 26) = 7.538, p = 0.006$. (visualized in Appendix II). We reason this difference could be due to reduced sensation, as although there was no significant difference between groups in tingling or sleepiness- a lack of sensation (e.g., numbing sensation) was not probed in this questionnaire. There was no significant differences in pitch counting across blocks between the groups ($F(21,1) = 0.141, p = .711$).

We used the same GLMM on response time during day 1 to compare the 20 Hz stimulation condition with and without topical anaesthetic. We found a significant effect of block ($p < 0.008$), but no significant effect of group ($p = 0.135$), nor interaction between group and block ($p = 0.999$) (Figure 4.10A). The same analysis was performed on day 1 accuracy, which also found a significant effect of block ($p < 0.001$) and group ($p = 0.002$), similar to the differences observed in experiment 1. The same skill GLMM used in experiment 1 found no main effect of group ($p = 0.992$) but a significant interaction between group and block ($p = 0.005$). Subsequent deviation analysis found no significant change in skill in the 20 Hz + lidocaine group across learning (all $p_{adj} > 0.05$) (Figure 4.10B). There was however significantly lower skill in the 20 Hz condition in mid-training ($p_{adj} = 0.001$), and significant greater skill in post-training ($p_{adj} = 0.019$). The GLMM of day 2 testing response time (Figure 4.10C) found a main effect of block ($p < 0.001$) and group ($p = 0.003$), but no main effect of hand ($p = 0.399$) nor any interaction. Similarly,

the GLMM of day 2 accuracy found only a main effect of block ($p < 0.001$) reflective of random-trial interference accuracy reduction (all contrast $p_{\text{adj}} < 0.05$).

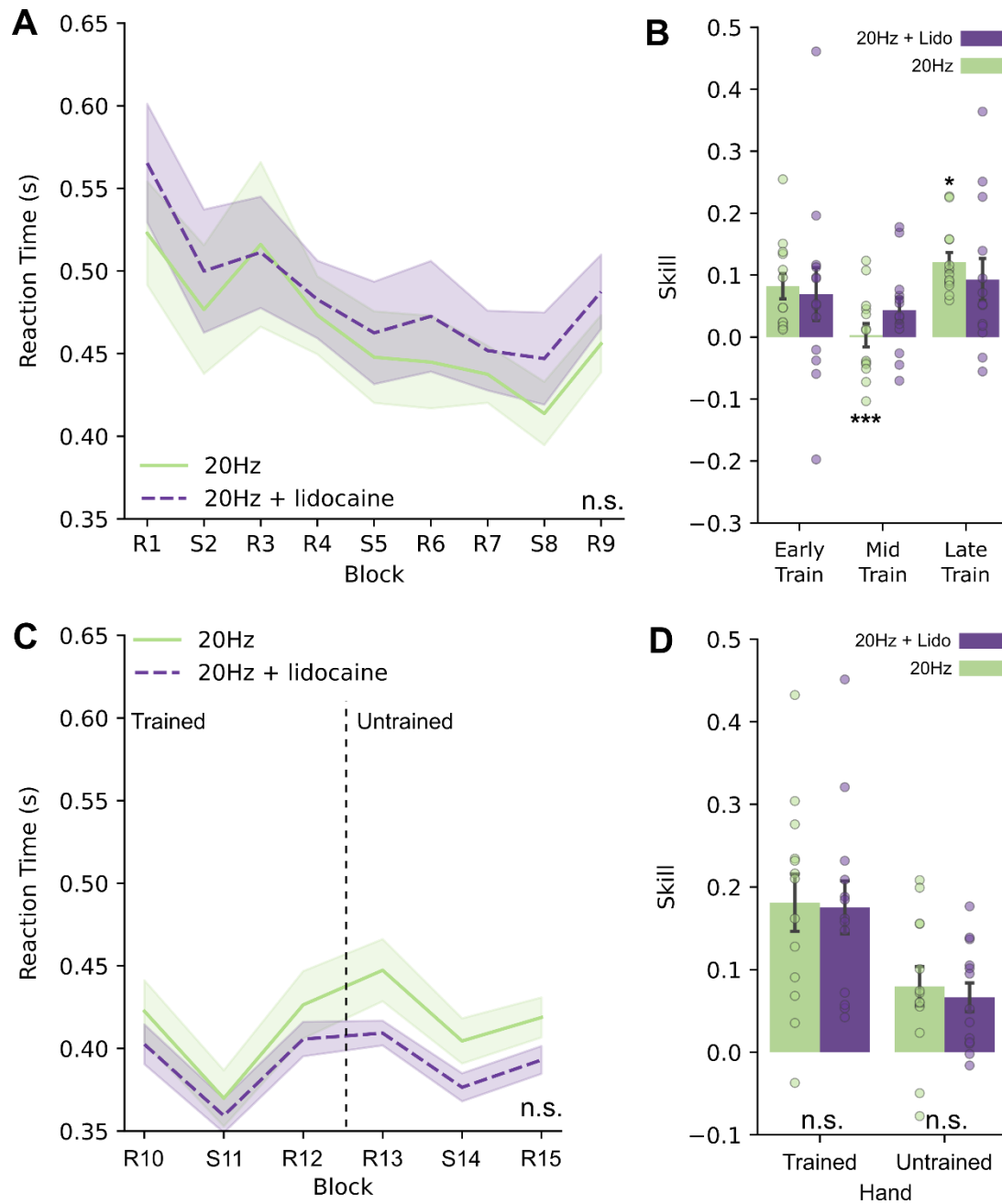


Figure 4.10 (A) Reaction time differences between 20 Hz group and 20 Hz with lidocaine. (B) Skill by training block. (C) Reaction time differences on day 2. (D) Skill by testing block. * $p < 0.05$, *** $p < 0.001$.

4.4 Discussion

Given the mixed findings in the field of tACS and motor learning, it is necessary to investigate how the role of peripheral mechanisms may contribute to the modulation of motor learning using transcranial/transcutaneous stimulation. Here, we combined electrical stimulation of the C2 dermatome, innervated by the greater occipital nerve, with an implicit motor sequence learning task to determine if transcranial stimulation would modulate motor learning. We selected 4 conditions; 2 control groups and 2 active groups, to determine if the effect is specific to a given frequency (20 Hz or 40 Hz), and significantly different from sham or 1 Hz stimulation parameters. We also used a 2-day approach, whereby participants returned 24 hours following training to determine if the effects observed on the first day persist into a second day, indicating longer-term changes. We looked mainly at two aspects of behaviour, the overall reaction time of performance, which would capture a mixture of task-general learning and sequence-specific learning, and the skill level, which measures sequence-specific learning relative to overall task performance (Robertson, 2007). We found that there was an effect of group on the overall reaction time during acquisition, where the 20 Hz group had significantly faster reaction time across the task on day 1 compared to the other groups. There was no significant difference in response time between either of the control groups, nor with the 40 Hz group. The raw skill and relative skill measures both found that the 20 Hz stimulation group displayed significantly greater skill than the other groups, indicating that not only was there improved task learning as shown by faster response times overall, but specifically improved implicit motor sequence learning. Difference in accuracy, however, may explain increased reaction time in the 20 Hz group. There was no significant difference between the groups in reaction time on the second day when participants returned, nor difference in trained or untrained hand skill. Thus, we found that 20 Hz stimulation specifically improves motor learning (both task and sequence), but the effects do not persist 24 hours later. We also found that 40 Hz stimulation, despite previous studies showing modulation of memory (Luckey, McLeod, Mohan, & Vanneste, 2022), and motor learning (Giustiniani et al., 2019a), did not modulate motor learning differently than either sham group nor active control.

This finding therefore suggests that 20 Hz stimulation of the occipital nerve appears to improve motor learning acquisition, replicates previous findings using beta-range stimulation directly to the M1 during motor learning (Pollok et al., 2015a), and potentially contributes to previous findings indicating that 20 Hz tACS may increase cortical excitability via an NMDA receptor-mediated mechanism (M. Wischniewski et al., 2019). While there was evidence of improved learning in the form of faster response times overall during stimulation (albeit concurrent with reductions in accuracy), and greater late-train motor skill, these effects did not carry into the following day, nor did they influence inter-manual transfer.

There are several reasons which could explain why improved motor acquisition did not result in behavioural differences in a retest period. One possible explanation is that the difference in task requirements between day 1 and day 2 sessions. During day 1, participants were asked to attend to an auditory stimulus and perform a concurrent counting task. This was designed to improve blinding against the sequence and ensure that motor learning was kept implicit (Jie et al., 2018). However, in order to better probe potentially small motor differences and avoid task compliance-induced variability, we opted not to add pitch counting to the day 2 task. Thus, it may be that a combination of overnight consolidation of the motor sequence (Debas et al., 2014; Debas et al., 2010), and reduced task demands on day 2 compensated any differences which arose during training on day 1, resulting in a ceiling effect (see figures 2.1 and 2.2 in **Chapter 2**).

Correlation analyses also revealed that while early-train skill levels did not predict late-train or day 2 testing skills, late-train skill levels (the final block of the day 1 training) correlated with the day 2 trained and untrained skill, and further, between the trained and untrained hand skill levels on day 2. These correlations, computed across all groups, indicate that existing early-training skill levels did not predict later skill acquisition, but that greater skill acquisition by the end of day 1 training correlated with better testing skill on day 2, suggesting against any influence of existing motor skill. We hypothesize that longer learning duration could better solidify the motor skill (Voelcker-Rehage, 2008), which may then translate to improved inter-manual transfer (Perez et al., 2007) (although it must be noted that other studies have suggested that learning

score does not significantly correlated with transfer scores, see Dirren et al. (2021)). Thus, in **Chapter 5** we will describe the second experiment which included additional learning blocks pre- and post-stimulation in order to better control for existing individual differences, and to determine if more learning heightens the effects which are prevalent in these findings (Nguyen et al., 2019), and improves inter-manual transfer levels.

In a secondary experiment we conducted an identical 20 Hz stimulation protocol with the addition of topical anaesthetic using a lidocaine/prilocaine cream. This approach has previously been used when investigating the validity of the peripheral hypothesis by preventing propagation of the stimulation via the nerve (Asamoah et al., 2019; Hermanns et al., 2019; Vanneste et al., 2020). Reaction times between the 20 Hz group and 20 Hz group + lidocaine did not significantly differ, which initially suggested that lidocaine did not prevent the acquisition modulation observed in the first experiment. We caution, however, that this experiment sought to observe the effect of peripheral nerve stimulation on motor learning, which we operationalize as skill metrics and degree of random-block interference. Looking at skill, we did observe a significantly greater skill level in the 20 Hz group compared to the 20 Hz + lidocaine group in the late-train block. This therefore suggests that despite a lack of differences in task speed, there was still greater learning prominent in the 20 Hz group, which was reduced with the addition of lidocaine. This is consistent with previous work using NITESGON and lidocaine in memory recall (Vanneste et al., 2020), and provides additional support for a transcutaneous mechanism. Given a lack of differences in the lidocaine and no-lidocaine group in acquisition, we also consider the possibility of some transcranial mechanism, particularly when considering the remaining three groups (2 controls, 1 active) did not show acquisitional modulation like the 20 Hz group. 20 Hz stimulation may indeed modulate acquisition transcranially, however without a pre-stimulation baseline, it is not possible to determine if differences in performance speed are existing or modulated aspects. This is discussed further in the limitations.

Taken together, the findings of this chapter are consistent with the combined theories of the LC as a global model failure system and of motor learning as an adaptive process engaging

an internal model, with NITESGON modulating learning via a peripheral mechanism. In light of the work which established 10 Hz (as opposed to 30 Hz) as an optimal LC stimulating frequency (Tseng et al., 2024) for motor plasticity, we found a similar result whereby neither 1 Hz nor 40 Hz stimulation produced the behavioural improvement observed in 20 Hz. Furthermore, following the adaptive learning model, LC activation at an optimal frequency would be proposed to induce plasticity via altering neural gain, and has indeed been shown to increase learning rates (Glennon et al., 2019), which we observe here. Pharmacological disruption of NE activity has been shown to reduced learning rates (Plewnia et al., 2004), and so there is evidence that the interaction between LC-NE activity and the adaptive motor learning processes fit with the theory behind NITESGON, and are supported by the behavioural findings here.

To control for sensation-driven or placebo effects, we administered questionnaires regarding sensations felt during stimulation, and the group-guess allocation. We found no significant difference between groups across sensation intensity reported during the stimulation, and no significant difference between group-guess and group allocation, however there was a trend whereby nearly 80% of individuals receiving 20 Hz stimulation believed they were in the active group, compared to 35%, 40%, and 50% in the other groups. Although not significant ($p > 0.05$), we cannot exclude the possibility that a placebo-effect driven by belief may have influenced some of the behaviour. It is unlikely however, given that behavioural effect was prevalent only during the day 1 acquisition, but not on the following day.

In the wider context of other work investigating 20 Hz tACS for motor learning, our results suggest that behavioural changes in motor learning, particularly during acquisition, are attainable via stimulation of the peripheral nerves, as opposed to delivery to the M1 region as has been reported previously (Bologna et al., 2019). Building on previous work, we hypothesize that the transcutaneous mechanism by which occipital nerve stimulation modulates motor learning could implicate the LC via the LC-NE pathway, subsequently altering cortical arousal and plasticity (Vanneste et al., 2020). Indeed animal studies investigating VNS paired with motor rehabilitation have found that nucleus basalis (NB) lesioning prevented VNS-driven cortical

plasticity, suggesting that acetylcholine may also be implicated in the observed behavioural modulation (Daniel R. Hulsey et al., 2016). Indeed, VNS has been shown to implicate both LC-driven noradrenergic and NB-driven cholinergic mechanisms, which may synergistically modulate neural plasticity (Rasmusson, 2000). A study by Hulsey et al. (2019) found that depletion of either noradrenaline or serotonin prevented cortical plasticity compared to controls, in favour of the theory that multiple neuromodulators are likely implicated in regulating synaptic plasticity. Thus, in the following chapter, we will introduce both neuroimaging in the form of resting-state and concurrent task EEG, and proxy measures of neurotransmitter activity via pupillometry, to better understand the neural changes underlying the observed behavioural modulation at 20 Hz, and explore the possibility of an LC-NE driven mechanism as has been proposed previously, particularly in the context of adaptive motor learning, and LC as a global model failure system (Luckey, McLeod, Mohan, & Vanneste, 2022; Vanneste et al., 2020).

There are several limitations that warrant consideration. First, we noted that despite blinding efforts and the presence of both active and passive controls, participant belief may contribute to the observed differences in behaviour. We also recognize that stimulation was delivered from the beginning of the training, and thus we lack a baseline measure to compare. Although our results indicate that the behavioural effect is the result of the stimulation, particularly after correcting for baseline skill in the first block, we recognize that a pre-stimulation baseline and post-stimulation follow-up would better control for existing differences. We also must consider the effect of handedness on these results, particularly as we did not exclude left-handedness, but rather instructed participants to perform the study with their non-dominant hand, thus while this improves generalizability of the findings, it also introduces variability via the effect of degree of handedness or ambidexterity (Chase & Seidler, 2008).

4.5 Conclusion

To conclude, **Chapter 4** illustrates that 20 Hz NITESGON during a motor learning task improves acquisition of the task, and motor sequence specific learning. The motor learning effect was subsequently reduced with the addition of lidocaine, suggesting a peripheral mechanism.

This finding is behaviourally consistent with modulated LC activity driving differences in learning rates, which have previously been associated with neurotransmitter modification, prediction error computation modulation, and subsequent plasticity (Jordan, 2024). This therefore establishes that NITESGON can be used to modulate motor learning acquisition via peripheral nerve stimulation and supports additional research to investigate further the potential mechanism and neural changes which result.

Chapter 5 The Effect of Peripheral Nerve Stimulation on the Electrophysiological Correlates of Motor Learning

5.1 Introduction

Given the established behavioural modulation achieved by NITEGON during a motor learning task, the next logical step in this study is to investigate biological correlates of these changes to understand mechanistically how NITEGON elicits its modulation. Following the consistency of the findings in **Chapter 4** with models of adaptive motor learning, modulation of cortical plasticity, and proof of a peripheral mechanism, here I pursue further investigation using methods of electrophysiological recording (EEG) and physiological arousal (pupillometry).

There are several established EEG measures of motor learning, which have ranged from microstate (Pirondini et al., 2017) to more general training (Jochumsen et al., 2017) correlates, although results still trend toward inconsistency in some ways. At the system level, changes in neural connectivity are established to influence motor learning. Increased alpha band and decreased beta band functional connectivity have both been found to predict learning (Mottaz et al., 2024; Titone et al., 2022). RsEEG analyses have also reported training-related changes in connectivity of numerous networks both within and outside of the motor network, including decreased gamma connectivity in the motor network, and decreased connectivity between motor-language, and motor-ventral attention networks (Titone et al., 2022). In a general sense, network connectivity changes in motor learning likely reflect learning-induced changes in connectivity relating to the recruitment or silencing of relevant and irrelevant brain regions respectively, and in more holistic terms, have been theorized to reflect global brain reorganization (Yu et al., 2020). Even small connectivity changes such as local connectivity measured via synaptic changes have been shown to occur during adaptation, with these changes possibly being driven by the cerebellum and parietal cortex, while higher order M1 and premotor cortices remain relatively the same (Feulner et al., 2022).

There are also temporally more distinct EEG responses which have been used to understand motor dynamics including the ERDS (desynchronization) and PMBR (resynchronization), both of which are potentials generated in alpha/beta range from the motor cortex and precentral regions during action (Heinrichs-Graham et al., 2017; Jurkiewicz et al., 2006; Takemi et al., 2013). The role of these potentials remains under debate, however there is evidence that PMBR may in part serve as motor inhibition following movement, and indeed larger PMBR is shown to predict longer preparation time for the subsequent movement (and slower reaction times), via inhibitory GABAergic pathways (Heinrichs-Graham et al., 2017; Zhang et al., 2024). Accordingly, it would be expected that with greater motor learning reflected by faster response times, reduced PMBR would accompany reduced inhibition of the subsequent movement (given an available prediction). There is however, contrary evidence suggesting that PMBR in fact attenuates as a result of larger error, placing PMBR as a reflection of the updating of internal models during motor adaptation (Tan et al., 2014). It is then likely a combination of factors which influence the magnitude of the PMBR, in which inhibition of the subsequent movement manifests as larger PMBR, while model updating attenuates this ERP (two processes which in theory would be either coincidental or sequential). Conversely, there is no clear functional role for ERDS as of yet, however it is believed to reflect several activities including sensory and cognitive processes relating to motor control, and has also been shown to negatively correlate with plasticity (Kilavik et al., 2013; Rau et al., 2003).

TACS is generally employed given its similarity to endogenous neural oscillations, and thus its utility for modulating oscillatory correlates of motor learning is fitting in this context. This may also provide some context as to why 20 Hz stimulation was most optimal for improving motor learning in **Chapter 4** compared to 40 Hz or 1 Hz, given that motor learning is commonly discussed within beta band activity (Barone & Rossiter, 2021; Espenhahn et al., 2020; Iwama et al., 2022; Koelewijn et al., 2008). This also synergizes with the proposal that the effect is driven by LC modulation, whereby 20 Hz is an optimal stimulating frequency to induce phasic firing in the LC, which further modulates the neural gain of predictive vs sensory input (Aston-Jones &

Cohen, 2005a, 2005b; Jordan, 2024). In fact, here we can also combine the correlates of motor learning in reduced PMBR, and the influence of the internal model in terms of LC neural gain framework. The theory puts forth that LC activity signals shift between stable internal models and rapid correction after model failure, and further that when relying on sensory as opposed to predicted input, that neuronal responses would be greater (Jordan, 2024). Thus, in early motor learning, we would expect that increased LC activity could alter neural gain and shift the learning to a primarily sensory input, which over the course of learning would reduce the neuronal response as a functional of internal model optimization and reliability. This would be hypothesized to occur as a combination of response magnitude reduction due to internal model reliance, and (concordant with behaviour) a reduction in neural inhibition and processing deemed unnecessary by the internal model. We expect that 20 Hz stimulation would improve learning and motor adaptation through this mechanism, which would be supported by reductions in motor response (potentially in the PMBR), and more accurate internal model (testable via re-test and inter-manual transfer (Wang & Sainburg, 2004)). Our experimental task is designed such that blocks switch between predictable sequence trials and unpredictable random trials. We can use these changes as a measure of susceptibility to interference (ultimately, motor skill), and also to observe the ability of individuals to ‘switch’ reliance from the internal model to external sensory information when the trial no longer follows the learned sequence. Jordan (2024) discusses how increased LC output increases learning and adaptation rates, altering the switch between sensory and model input. Thus, we may expect to observe behavioural or electrophysiological correlates of altered neural gain in both neural response and reaction time and accuracy following task demand changes.

To further validate the involvement of the LC, given that direct recording in humans is not presently possible, LC activity is instead indexable to some extent using pupillometry (Murphy et al., 2014; Reimer et al., 2016; Vanneste et al., 2020). Pupil diameter (dilation) has been shown to be an accurate tracker of fluctuations of both Ach and NE cortical activity (Reimer et al., 2016), and more directly has been shown to covary with LC activity (Murphy et al., 2014),

a relationship present at rest, and during task performance. The methodology can then be used to approximate the state of the LC-NE system *in vivo*. The behavioural evidence for the LC as a global model failure system also includes reductions in associative learning rates with pupil dilation changes, corroborating its use in behavioural paradigms seeking to investigate the LC in that role (Jordan, 2024), as we do here.

In this chapter, I therefore wish to test the hypothesis that the behavioural improvements observed during 20 Hz NITESGON in **Chapter 4** possess neural correlates which may support modulation of the adaptive motor learning processes. This will be carried out using both EEG and pupillometry before and after the task to observe changes in resting state functional connectivity and pupillometry, as well as task-related changes in processes via PMBR and ERDS. Modifications to the task length, which now include a baseline and follow-up block, will also allow for greater understanding of the effect of NITESGON on behavioural modulation by considering pre-stimulation existing differences between groups.

5.2 Methods

5.2.1 Participants

30 participants recruited to participate in the study (mean age = 21.600 years, SD = 1.812 years, range = 18-26 years). Participants were required not to have any tES contraindications, and no neurological or psychiatric diagnoses. The beck depression inventory (BDI) and beck anxiety inventory (BAI) were used to exclude individuals above a moderate/severe category of depression (>15) or anxiety (>18). Handedness was assessed using a short form version of the Edinburgh Handedness Inventory (EHI). Informed consent was given prior to participation. Participants were randomly assigned to either sham or 20 Hz stimulation groups. The sham condition had n = 15, mean age = 21.667 years, SD = 1.407 years (15F, 0M). The 20 Hz condition had n = 15, mean age = 21.553 years, SD = 2.193 years (12F, 3M). Handedness was similar between groups (sham: 3L, 12R, 20 Hz: 4L, 11R). More detailed demographic data are presented for completeness in Appendix III.

5.2.2 Experimental procedure

Experimental procedure followed a similar paradigm as **Chapter 4**, however with notable modifications. The paradigm was carried out as originally over 2 days. On day 1, there was a training session where individuals received active or sham stimulation and completed a version of the SRTT with their non-dominant hand, and on day 2 participants returned to perform 1 block of the same task with each of their trained (non-dominant) and untrained (dominant) hands. In this chapter, we altered the task to include additional training blocks to better understand the pre- and post-training effects. Thus, participants in this study performed 5 blocks of training; 1 block of baseline, 3 blocks with concurrent stimulation/sham, and 1 block post-train. Participants were still asked to count high tones during the training as in **Chapter 4**. Furthermore, participant's EEG was also recorded before, during, and after training on day 1, and during testing on day 2. RsEEG was recorded before and after training on day 1 with pupillometry, and EEG was also recorded while participants completed the task (more detailed procedures are described in 5.2.4). All other task parameters and instructions remained the same. A schematic of this paradigm is shown in Figure 5.1.

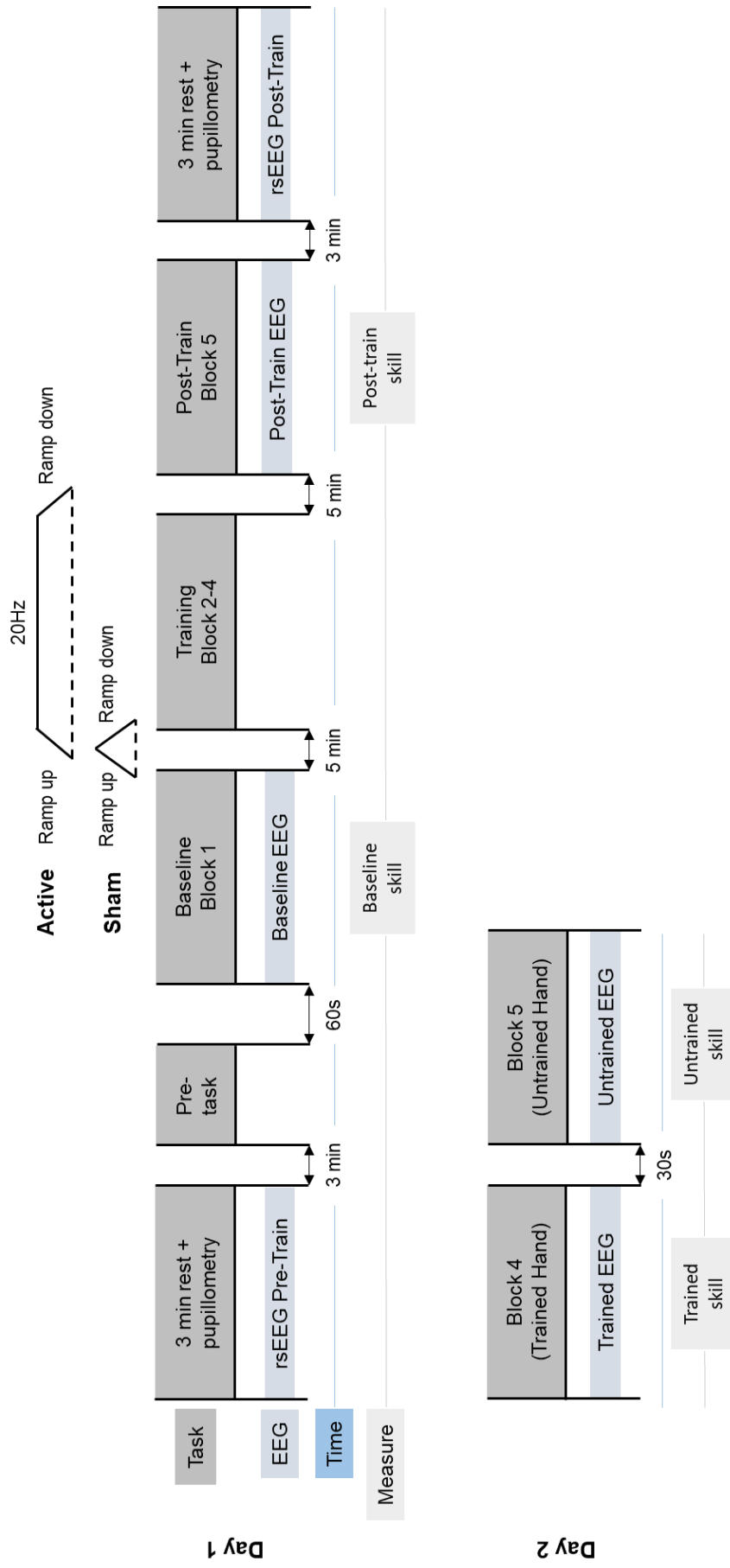


Figure 5.1 Schematic of experimental paradigm across training and testing days.

5.2.3 Stimulation protocol

Stimulation was delivered to the C2 dermatome using a DC-Stimulation Plus device (NeuroConn, Germany). Saline-soaked electrodes (35cm²) were placed with anode on the left side of the dermatome, and cathode on the right, wires facing inward (more detailed figure in Figure 4.3 and Figure 5.2). During the sham stimulation condition, stimulation delivered in a sinusoidal wave was ramped up over 30 seconds to 1.5mA at 1 Hz frequency, which lasted 60 seconds, followed by a 30 second ramp-down, before the participant was told to begin the task. For the 20 Hz condition, stimulation was delivered for the duration of the training session (~25 minutes, 5 mins $\times$ 5 blocks) at 1.5mA at 20 Hz, following a ramp-up of 2 seconds. Participants were instructed to begin after 30 seconds to allow time for complete ramp-up. The 20 Hz/sham stimulation montage was set-up prior to beginning the baseline block (following rsEEG) in order to acquaint participants with electrode and securing band. Stimulation was delivered after the baseline block and ended before the post-train block. Participants then completed the post-train block. Only after this final block did the participants have the electrodes and band removed, before continuing with the post-train rsEEG. This was done in order to reduce the amount of interruption during training blocks.

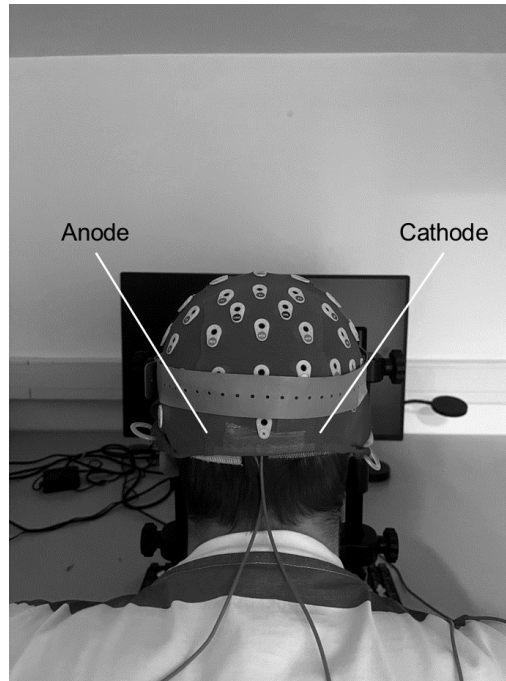


Figure 5.2 Example of concurrent tES and EEG cap (recording electrodes not shown for placement clarity). Stimulating electrodes were placed approximately underlying O1 and O2 recording electrodes (removed to prevent damage from induced electrical current). Participant is seated with head immobilized using forehead and chin rest.

5.2.4 EEG acquisition

EEG (both resting state and concurrent with task) were acquired via 64 Ag/AgCl electrodes in standard 10-20 montage using a Biosemi ActiveTwo system (Amsterdam, Netherlands). Data were collected at 4096 Hz and processed offline. All data were collected in a darkened room with participants seated comfortably. Prior to participation, participants were instructed to avoid caffeine and nicotine directly before the session. Participants were asked to avoid excessive eye movement, jaw movement, and crossing of arms and legs. During rsEEG, participants were asked to sit with their head immobilized in a chin and head rest. Participants were asked to sit with their eyes open, focusing on a fixation cross in the centre of a 60cm × 34cm monitor with 75 Hz refresh rate and 1024×768 resolution. Participants sat approximately 60 cm from the screen. RsEEG was recorded for 3 minutes prior to the task, and 3 minutes following the task. Eye tracking concurrent with rsEEG was recorded using Eyelink 1000 Plus eye tracker (SR Research Ltd., Ottawa, ON, Canada) with sampling rate 1000 Hz. Tracking recorded the

right eye of the participant and measured horizontal and vertical position of the pupil, along with pupil size via ellipse fitting.

Concurrent task and EEG was carried out without eye tracking as participants were not immobilized to allow more comfortable posture during the task. Participants completed the task as in **Chapter 4**, and EEG was continuously recorded during baseline and post-train blocks. Data were also collected concurrent with stimulation, however stimulating electrode placement disrupted the reference electrode's function, preventing further analysis.

5.2.5 Analysis

Due to external factors (equipment malfunction, participant error), exclusion of certain participants was necessary for certain analyses. In order to conserve maximal sample size, participant data were used where available, as opposed to list-wise or case-wise deletion. Sample sizes or degrees of freedom are clearly stated for each analysis, and for clarity the participant exclusion numbers are presented in Appendix III.

5.2.5.1 Behavioural analysis

Behavioural data (i.e., reaction time, accuracy) were preprocessed in the same manner as in **Chapter 4**. A GLMM was used to analyze the effect of block and group on reaction time. Block (baseline, training 1-3, and post-training), and trial type (sequence and random) were included as repeating measures. Group was included as the between subject factor, and reaction time was the outcome measure. A GLMM was used to analyse the effect block on skill, as calculated in Equation 4.1. Group differences in baseline and post-training skills were individually tested using t-tests, and training blocks 1-3 were analysed using the GLMM, where block was included as the repeating measure, skill as the outcome variable, and group as the between subject factor. As a post-hoc analysis, we also performed ANOVAs on each of the Sequence-Random interference pairs, to determine if interference differentially affected the groups. We considered this as a secondary analysis which more generally looks at performance overall as opposed to the computed skill measures, given the additional training (baseline block)

and consolidation periods which were added from experiment 1. Post-hoc testing was carried out to determine if there was a significant difference in reaction time between sequence and the interfering random block for each group, with Bonferroni correction.

5.2.5.2 EEG analysis

Day 1 rsEEG, day 1 EEG + task, and day 2 EEG + task were all preprocessed using EEGLAB using the same procedure. Recordings were down sampled to 500 Hz and re-referenced to the average. Data were band-pass filtered using a basic finite impulse response (FIR) filter, lower edge = 0.55 Hz and upper edge = 44 Hz. Data were plotted to visually inspect for noise channels, which were interpolated where necessary. Time intervals during rsEEG where data contained artifacts (e.g., muscle, high-frequency noise) were manually selected and removed. EEG + task were epoched using the onset of the button press as the event and included 400ms preceding and 400ms following the event. Epochs were removed if they contained artifacts. Data were then decomposed using independent component analysis (ICA) using Infomax runica with maximum 64 components, adjusted for the number of channels removed ($N_{\text{Component}} = 64 - N_{\text{Interpolated}}$). Components were manually selected and removed if they included signal representing eye-movement or blinking. Components were not removed if they were localized over ROIs (e.g., central, occipital). All participants were included for rsEEG, and day 1 EEG + task. For day 2 EEG, 3 participants were removed during preprocessing, 2 due to technical error during recording leaving $n = 13$ in the 20 Hz stimulation group and $n = 15$ in the sham stimulation group. In one participant, the untrained-hand EEG was not recorded, and so analyses of untrained hand EEG were performed with $n = 12$ in the 20 Hz group, and $n = 15$ in the sham group.

Data analysis was performed with Brainstorm (Tadel et al., 2011), which is documented and freely available for download online under the GNU general public license. During rsEEG, lagged phase coherence, individual beta frequency (iBF), and power analyses were performed. Peak beta frequency (individual beta frequency (iBF)) was determined during pre- and post-training rsEEG. A fast Fourier transform (FFT) was applied to continuous data filtered between 15-29 Hz (beta range). The peak power (max) over the contralateral electrode (C1 or C2) was

identified and its corresponding frequency was selected as the iBF for that individual (Baumgarten et al., 2016). The same processes were performed on the ipsilateral side, before and after training.

RsEEG power analysis was performed in the beta range during the pre- and post-train by first decomposing rsEEG data for each individual into a time-frequency representation, extracting the beta range (15-29 Hz) power, and then averaging across participants by group to produce time $\times$ group cortical plots of mean beta power. Difference plots were produced by subtracting post-pre beta power within subjects, and averaging difference plots within groups. We selected two electrode sets, the first representing power differences in the motor cortex (contralateral M1, C1/C2), and the second set representing power differences under the stimulation electrode locations (i.e., PO3, PO4, O1, O2). Mean power at these sites was then compared between groups using an independent samples t-test.

RsEEG connectivity analysis was performed on continuous resting state data using lagged-phase coherence between electrodes in the M1 regions (C1, C2, C3, C4). Lagged phase coherence was estimated using a window overlap of 0.5, with Morlet wavelet time-frequency decomposition (central frequency = 1 Hz, time resolution (FWHM) = 3s), across all frequencies (i.e., 1-60 Hz) and then beta coherence was extracted and averaged across 15-29 Hz. Interhemispheric connectivity was then calculated as the mean coherence between left and right hemispheric electrodes (i.e., C1-C2, C1-C4, C3-C2, C3-C4).

During active task and EEG, ERPs were analyzed. For each participant, trials were average by condition and hand (sequence and random trials on both the trained and untrained hand). For each trial $\times$ condition combination, event-related beta desynchronization (ERDS) and event-related synchronization (ERS) were computed by first using a Morlet wavelet transform in the beta range (13-29 Hz) to generate a beta-power $\times$ time representation at each sensor. For each individual, the minimum power preceding the button press (defined between -200ms and 0ms, where 0ms is the button press event), was considered the beta-desynchronization value. Similarly, the maximum power following the button press (defined between 0ms and 200ms) was

considered the event-related synchronization (Sehatpour et al., 2023). Contralateral and ipsilateral M1 was defined simply as data from electrodes C1 and C2, depending on handedness of the participant (e.g., when using the left hand for the task, contralateral M1 was defined as sensor C2, and ipsilateral as C1). This was selected given that we are most interested in activity relating strictly to hand/finger movement, and previous research has shown that, despite many studies selecting C3/C4, MEPs recorded in the left and right hand were strongest when single-pulse TMS was delivered the C3h and C1 areas, as opposed to C3 (Kim et al., 2023). Therefore, we pursued analysis of C1/C2 when investigating neural correlates of motor learning in a hand-based task.

Pupillometry was preprocessed first by plotting pupil size over time (180s), and automatedly removing periods where pupil was not tracked (e.g., blinks, extreme deviation). Maximum and minimum pupil size were then identified via global max and global min functions in Matlab (MATLAB 2022b). The difference between maximum and minimum pupil size (in arbitrary units) was then determined for each individual pre- and post-training.

5.2.5.3 Statistical analysis

Mean iBF was determined for each group at each time point, and a GLMM was performed to determine if there was a significant change in iBF from pre- to post-train within each group. Mean interhemispheric connectivity was calculated for each group and correlated with skill measures using a Pearson correlation. Pupil dilation range differences were calculated using a GLMM with factors for group and time determine if there was a significant change in pupil dilation range from pre- to post-training.

Day 2 ERDS/PMBR were analyzed in Jasp (JASP, 2024). A GLMM was used to analyze the interaction between group and hand for each of the sequence and random trials on contralateral and ipsilateral motor regions. Correlation analysis between even-related potentials and behavioural measures was performed using a Pearson correlation. A significant result was considered $p < 0.05$, with corrections where stated. Group-averages for each contrast (hand $\times$ trial type $\times$ hemisphere) were computed by average ERDS and PMBR values accordingly. Thus, the

terms ‘contralateral’ and ‘ipsilateral’ here do not generally indicate left or right hemisphere, since handedness varied across participants. One-sample t-tests were used to determine if there was a significant change from baseline (0% change).

5.3 Results

5.3.1 No sensory or task compliance difference across between groups

A t-test was performed to compare the mean sensation reported for the sham and 20 Hz group based on 10 different sensations (Table 5.1). Pre-correction, only tingling was reportedly stronger in the 20 Hz group compared to the sham group, however after correction for multiple comparisons this result was non-significant. For further validation, we chose to pursue an additional ANOVA whereby the participant's group-guess was used as a factor and tingling as the outcome variable, there was no significant effect of group-guess on tingling severity ($F(1,26) = 0.002, p = .964$), and so we may inversely infer that although those receiving 20 Hz stimulation reported marginally greater tingling, this relationship did not transfer to belief of group allocation.

Table 5.1 Mean sensation intensity for each group following stimulation with T-test results.

Sensation	Mean sensation $\pm$ SD ^a		T-test		
	Sham	20 Hz	<i>T</i> (28)	<i>p</i> _{uncorr}	<i>p</i> _{corr} ^b
Headache	1.267 $\pm$ 0.458	1.267 $\pm$ 0.458	1.474	.152	1.00
Neck Pain	1.067 $\pm$ 0.258	1.067 $\pm$ 0.258	0	1.000	1.00
Scalp Pain	1.000 $\pm$ 0.000	1.143 $\pm$ 0.535	-	-	-
Tingling	1.200 $\pm$ 0.414	1.800 $\pm$ 0.676	2.931	.007	.07
Itching	1.133 $\pm$ 0.352	1.500 $\pm$ 0.650	1.906 ^c	.067	.67
Burning	1.067 $\pm$ 0.258	1.333 $\pm$ 0.617	1.544	.134	1.00
Skin Redness	1.000 $\pm$ 0.000	1.000 $\pm$ 0.000	-	-	-
Sleepiness	2.000 $\pm$ 1.533	1.533 $\pm$ 0.640	-1.606	.120	1.00
Trouble Concentrating	2.133 $\pm$ 1.060	1.600 $\pm$ 0.828	-	-	-
Acute Mood Changes	1.133 $\pm$ 0.516	1.000 $\pm$ 0.000	-1.536	.136	1.00

^a1 = absent, 2 = mild, 3 = moderate, 4 = severe.

^bBonferroni corrected for 10 comparisons.

^c*T*(27) due to absence of one response.

There was no effect of group on the reported number of pitches counted across the 5 blocks ($F(1,27) = 0.198, p = .660$), and all participants reported normal sleep between day 1 and day 2. The proportion of participants guessing the active/non-active condition did not differ by group $X^2(1, N = 30) = 0.186, p = 0.666$ (Figure 5.3).

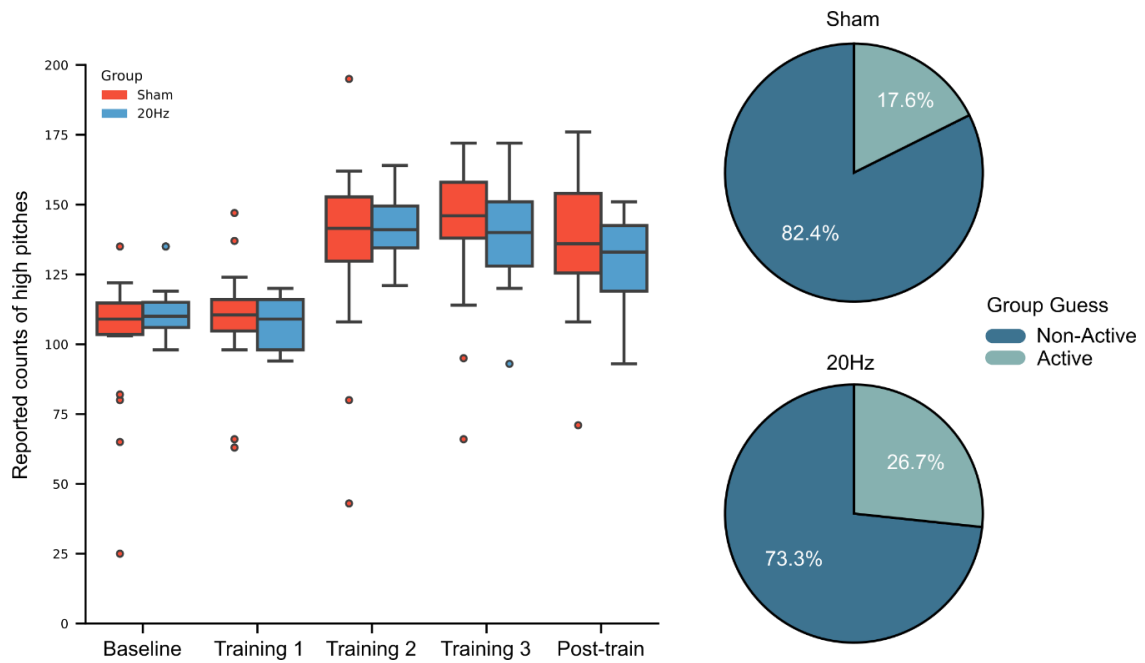


Figure 5.3 Reported counts for each block between groups, and group guess for the sham (upper) and 20 Hz group (lower).

5.3.2 20 Hz stimulation modulates motor skill acquisition during task

The rmANOVA on subblock ($n=15$) found a significant effect of sub-block on reaction time ($F(14) = 28.715, p < 0.001$ (Greenhouse-Geisser corrected)), but no significant interaction between sub-block and group ($F(1,14) = 0.766, p = .52$ (Greenhouse-Geisser corrected)). The rmANOVA on block ($n=5$) revealed a main effect of block on reaction time ($F(4) = 39.974, p < 0.001$ (Greenhouse-Geisser corrected)). There was no main effect of group ($F(1) = 0.0007, p = .98$), nor significant interaction between block and group ($F(1,4) = 0.513, p = .59$ (Greenhouse-Geisser corrected)) (Figure 5.4A). There was no difference in skill during the baseline block between groups ($t(28) = 0.203, p = .84$). The rmANOVA on skill of training blocks 1-3 revealed a significant effect of training block on skill ($F(2) = 5.11, p = .009$), but no effect of group ($F(1) = 0.036, p = .851$), and no significant interaction between group and block ($F(1,4) = 1.222, p = .302$). There was no difference in skill during the post-training block between groups ($t(28) = 0.201, p = .84$) (Figure 5.4B). An rmANOVA on accuracy revealed a significant effect of block on accuracy ($F(14) = 3.017, p < 0.001$), but no main effect of group ($F(1) = 2.655, p = .114$), and no interaction between block and group ($F(1,4) = 1.197, p = .275$) (Figure 5.4C). There was, however, a significant interaction between trial and group ($F(1,2) = 3.206, p = .048$), and further post-hoc analysis found that the sub-block did not differ for the initial random trials ($t(46.108) = -1.118, p_{corr} = .807$) nor the sequence trials ($t(46.108) = -0.533, p_{corr} = 1.00$), but did significantly differ for the interfering random trial across all blocks ($t(46.108) = -2.613, p_{corr} = .036$; estimated difference in accuracy = -0.024 , $SE = 0.009$). More directly, this indicates that the sham group had significant reductions in accuracy compared to the 20 Hz group when trials changed from sequence to random. The results of the secondary reaction time analyses which considered the effect of interference trials is presented in Table 5.2.

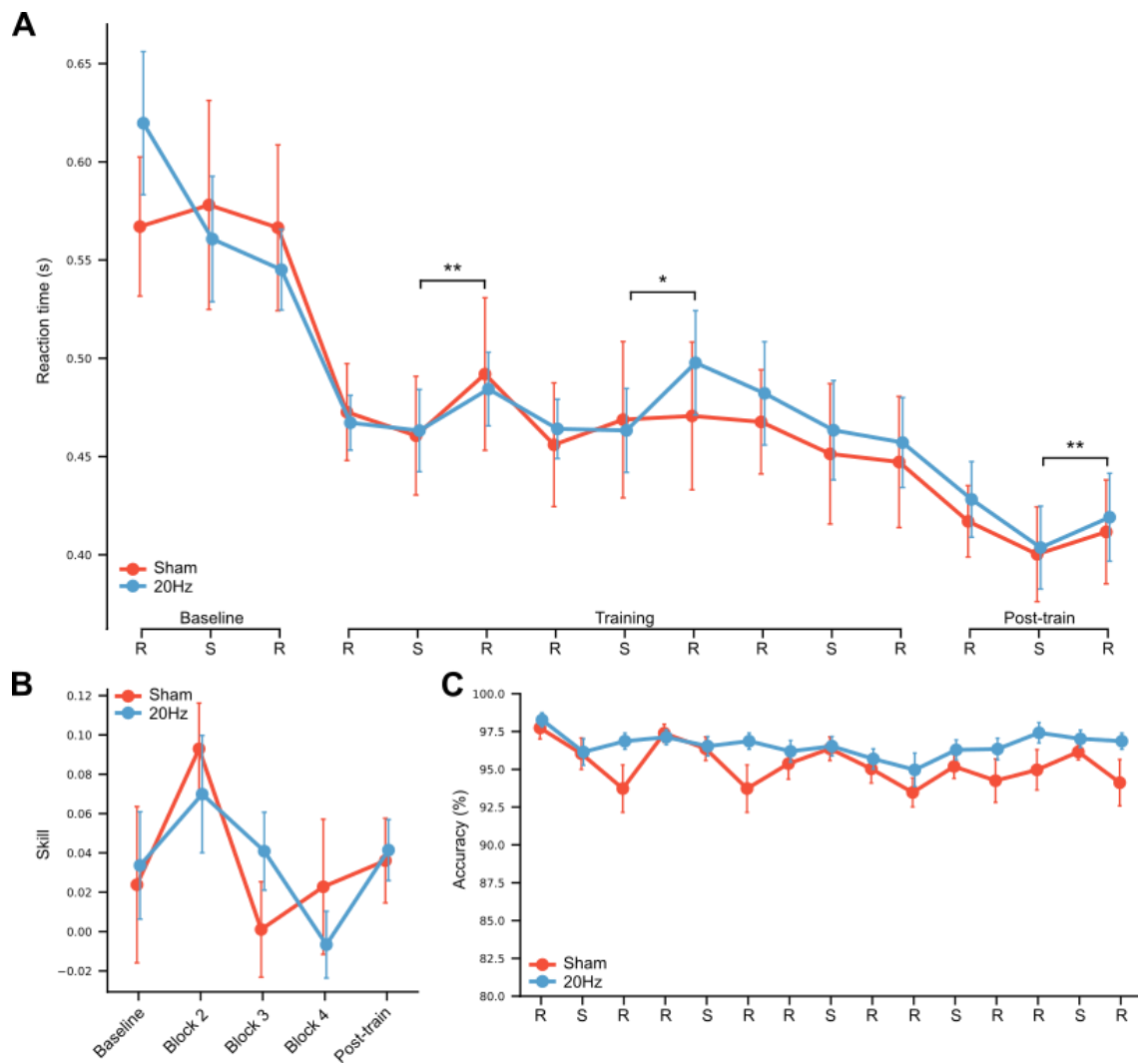


Figure 5.4 A) Reaction time (s) over training blocks on day 1. Baseline blocks occurred before stimulation, training blocks during stimulation, and post-train blocks following stimulation. R and S denote random and sequence trial blocks respectively. B) Skill by group for each block during training. C) Accuracy over training blocks on day 1. All error bars indicate SEM, colour denotes group. * $p < 0.05$ ** $p < 0.01$.

Table 5.2 Secondary analysis of sequence and interfering random trials during day 1 training.

Block	ANOVA Results			Post-hoc testing ^a	
	Trial	Group	Trial $\times$ Group	Sham	20 Hz
Baseline	F = 0.676 <i>p</i> = .418	F = 0.136 <i>p</i> = .715	F = 0.015 <i>p</i> = .904	<i>t</i> = 0.496 <i>p</i> _{tukey} = .959	<i>t</i> = 0.667 <i>p</i> _{tukey} = .908
Training 1	F = 9.582 <i>p</i> = .004	F = 0.004 <i>p</i> = .949	F = 0.363 <i>p</i> = .552	<i>t</i> = 2.615 <i>p</i> _{tukey} = .064	<i>t</i> = 1.763 <i>p</i> _{tukey} = .312
Training 2	F = 5.159 <i>p</i> = .031	F = 0.058 <i>p</i> = .812	F = 4.135 <i>p</i> = .052	<i>t</i> = 0.168 <i>p</i> _{tukey} = .998	<i>t</i> = 3.04 <i>p</i>_{tukey} = .030
Training 3	F = 0.554 <i>p</i> = .463	F = 0.070 <i>p</i> = .794	F = 0.023 <i>p</i> = .881	<i>t</i> = 0.419 <i>p</i> _{tukey} = .975	<i>t</i> = 0.633 <i>p</i> _{tukey} = .920
Post-Training	F = 9.77 <i>p</i> = .004	F = 0.027 <i>p</i> = .871	F = 0.224 <i>p</i> = .64	<i>t</i> = 1.87 <i>p</i> _{tukey} = .26	<i>t</i> = 2.546 <i>p</i> _{tukey} = .074

^a Comparing sequence to random reaction time, corrected for comparing family of 6.

5.3.3 20 Hz stimulation improves inter-manual skill transfer after 24 hours

A GLMM of the day 2 re-test response time data (Figure 5.5) revealed a significant effect of hand ($p = 0.002$) and block ($p < 0.001$). By block, there was a significant negative deviation in response time from the mean ($p_{\text{adj}} < 0.0001$) during the sequence block, while the trained hand had significantly reduced response times compared to the untrained hand ($p_{\text{adj}} = 0.002$). Thus, there was overall evidence of sequence learning retained 24 hours later, with better learning on the trained hand.

To further understand persisting effects, we carried out a GLMM analysis to understand the effect of stimulation on hand transfer of skill^{31,32}. We found a significant interaction between group and the hand used on skill ($p = 0.004$), in which the 20 Hz group had only a weak change in skill when transferring hands ($p_{\text{adj}} = 0.075$, contrast estimate: 0.074), while the sham group had a significant reduction in skill ($p_{\text{adj}} = 0.002$, contrast estimate: 0.134) (Fig. 4e).

These results suggest that with only slight improvement in acquisition and accuracy during training, 20 Hz stimulation improved inter-manual transfer ability, a metric of motor learning and a useful clinical feature³² in stroke recovery.

We reasoned that if the 20 Hz group did not have a significant decrease in skill following hand transfer, that the inter-manual transfer index would be closer to 0, indicating less skill loss, and thus a 1-sided t-test was used to test if the 20 Hz group had a significantly lower transfer index compared to the sham group. We confirmed this hypothesis, as the 20 Hz group had a significantly lower transfer index ($t_{1\text{-sided}}(28) = 2.005, p = .027$), with a mean transfer index = 0.138, SD = 0.675, while the sham group had a mean transfer index = 0.617, SD = 0.634 (Figure 5.5C).

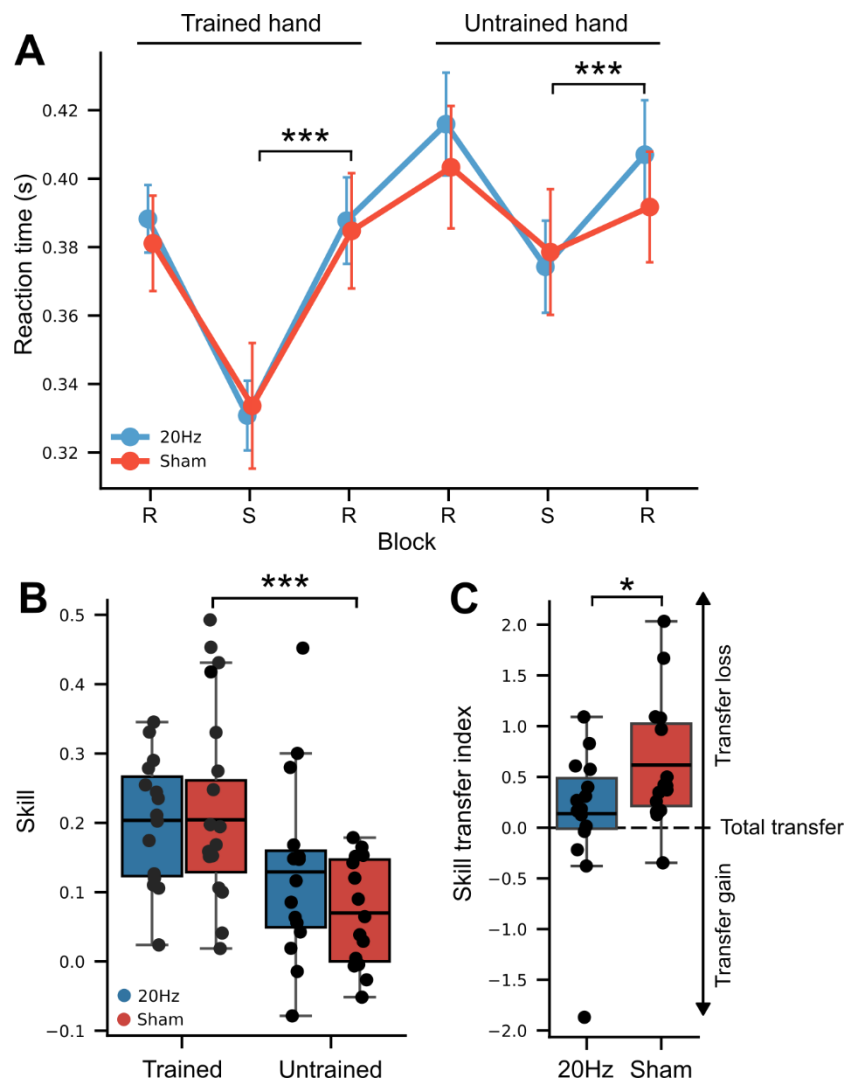


Figure 5.5 A) Reaction time over testing blocks on day 2. R and S denote random and sequence trial blocks respectively. B) Skill for each of the trained and untrained hand. C) Skill transfer index for each group, where 0 indicates no difference in skill between hands, a more positive number indicates loss of skill, and a more negative number indicates greater skill on the untrained hand. Colour denotes group, and all error bars denote standard error of the mean. Boxplots are overlaid with individual datapoints. * $p < 0.05$, *** $p < 0.001$.

5.3.4 20 Hz stimulation modulates resting connectivity correlated with untrained skill transfer

We first investigated resting state connectivity pre-train and post-train across both groups. Interhemispheric connectivity in the motor cortex pre-train and post-train was entered in an GLMM with factors for group and its interaction with time found an effect of group ($p = 0.044$) and interaction with time ($p = 0.012$). The 20 Hz group displayed a significant decrease in connectivity over training (contrast estimate: -0.045 , $p_{\text{adj}} = 0.012$), while the sham group did not display the same strong change in connectivity (contrast estimate: -0.028 , $p_{\text{adj}} = 0.094$) (Figure 5.6B).

We then wished to determine if connectivity changes between left and right motor cortices predicted subsequent behavioural modulation, and so performed a Pearson correlation between the post-train connectivity on day 1, and the performance (skill) of the untrained hand on day 2 (congruent with the observation that behavioural group differences were prominent in hand-transfer, specifically in skill on the untrained hand). Correlation analysis found a significant negative correlation between Post-train interhemispheric connectivity in the beta band, and untrained-hand skill ($r = -0.407$, $p = .035$, $n = 27$), such that lower connectivity correlated with greater skill (Figure 5.6A).

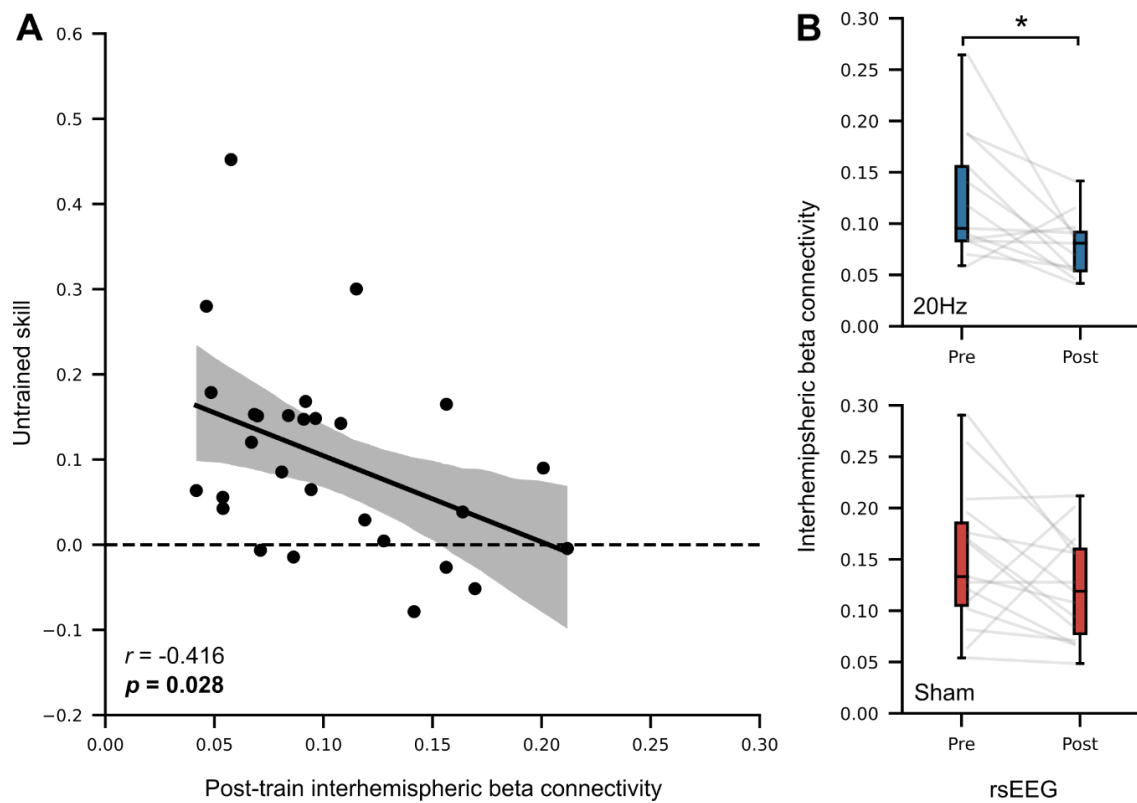


Figure 5.6 A) Interhemispheric beta connectivity across the motor cortex and untrained-hand skill (day 2) correlation. B) Interhemispheric beta connectivity pre-train and post-train for the 20 Hz (upper) and sham (lower) groups. Individual data are indicated by grey line. Boxplot includes median line. $*p_{\text{corr}} < 0.05$. Shaded area denotes 95% confidence interval.

5.3.5 Modulated PMBR reflects motor skill and sequence acquisition

Learning (sequence) related ERPs are shown for each timepoint in Figure 5.7A and 5.7B. We individually analyzed contralateral and ipsilateral neural activity. Contralateral visualization of ERPs revealed a decrease in PMBR from pre-train to post-train, and thus we pursued analyzing if this reduction in PMBR was significant, and specific to learning-related trials. We analyzed the contralateral PMBR by time-point between groups, individually for random and sequence trials (Figure 5.7C-F). Where data did not violate normality, a paired t-test was used, and otherwise a Wilcoxon signed-rank test was used. A Bonferroni correction is applied for a family of 6 contrasts. In the sham stimulation group, specifically for the sequence trials, there was a

significant difference between pre-train PMBR (mean = 3.828E-15, SD = 2.391E-15) and post-train PMBR (mean = 1.995E-15, SD = 1.339E-15) ($t(14) = 3.549$, $p_{\text{corr}} = .018$). There was no difference between pre-train PMBR and trained hand day 2 PMBR ($z = 1.193$, $p_{\text{corr}} = 1.00$) nor untrained hand day 2 ($z = 1.931$, $p_{\text{corr}} = .33$). There was no difference between post-train and trained day 2 hand PMBR ($z = -1.477$, $p_{\text{corr}} = .906$), nor untrained day 2 hand PMBR ($t(14) = -1.127$, $p_{\text{corr}} = 1.00$). There was no difference in PMBR between trained and untrained hands on day 2 ($z = 0.284$, $p_{\text{corr}} = 1.00$) (Figure 5.7C). There was no significant difference in any random trial contrasts (see Appendix III for results) (Figure 5.7E).

In the 20 Hz group, specifically for the sequence trials, there was a significant decrease in PMBR from pre-train (mean = 5.757E-15, SD = 5.396E-15) to post-train (mean = 2.447E-15, SD = 1.925E-15) ($z = 2.953$, $p_{\text{corr}} = .012$), and from pre-train to trained day 2 hand (mean = 2.025E-15, SD = 1.167E-15) ($z = 2.760$, $p_{\text{corr}} = .018$). There was no significant difference between pre-train and untrained day 2 hand PMBR ($z = 1.334$, $p_{\text{corr}} = 1.00$). There was no significant difference between post-train and trained hand day 2 ($t(12) = 0.620$, $p_{\text{corr}} = 1.00$), nor untrained hand day 2 ($t(11) = -1.160$, $p_{\text{corr}} = 1.00$). There was no difference in PMBR between hands on day 2 ($t(11) = -2.417$, $p_{\text{corr}} = .204$) (Figure 5.7D). There were no significant differences in any contrasts in the random trials (see Appendix III for results) (Figure 5.7F).

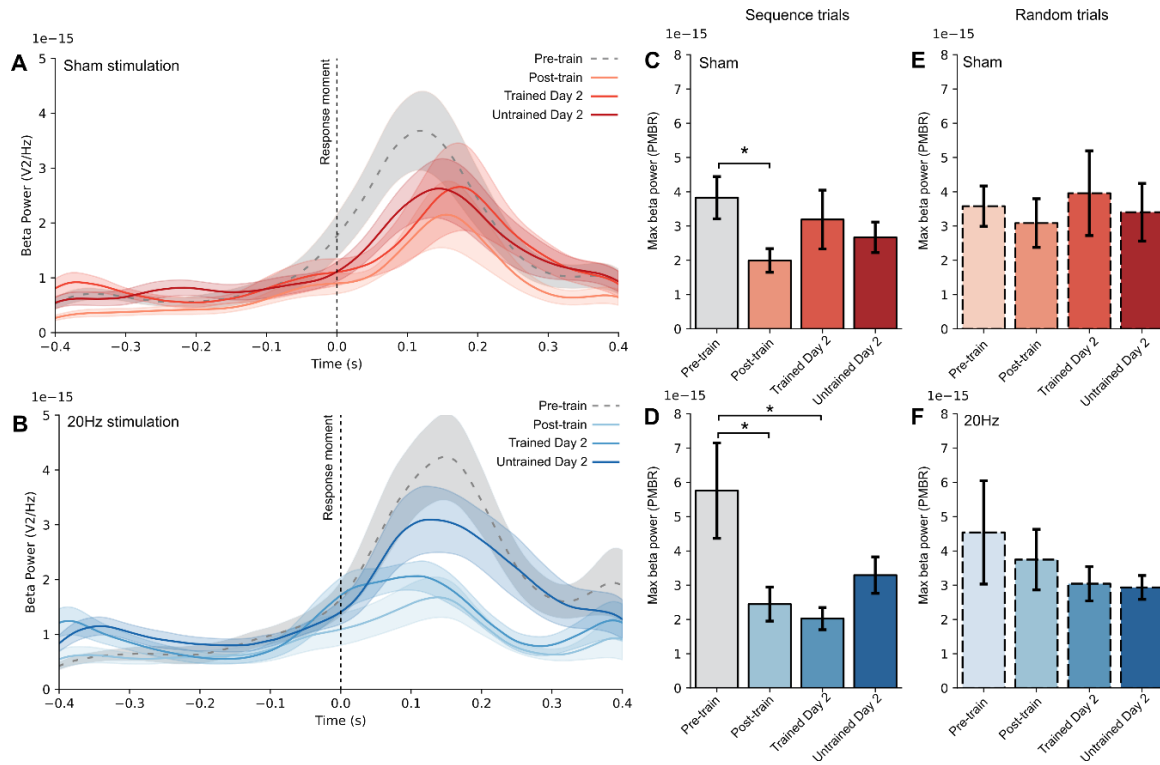


Figure 5.7 Contralateral beta activity and PMBR A) Sham stimulation and B) 20 Hz stimulation beta activity during sequence trials over the contralateral M1. C) Sham stimulation and D) 20 Hz stimulation PMBR during sequence trials at each time point. E) Sham stimulation and F) 20 Hz stimulation during random trials at each time point. * $p < 0.05$. (Bonferroni corrected).

We repeated this approach on the ipsilateral side, initially visualizing beta band activity over the ipsilateral M1 during sequence trials across time points to observe a learning effect (Figure 5.8A and 5.8B). We then performed comparisons within each group and time-point, correcting for 6 comparisons. Where data did not violate normality, a paired t-test was used, and otherwise a Wilcoxon signed-rank test was used. In the sham stimulation group, there was no significant difference between pre-train and post-train PMBR on the ipsilateral side during sequence trials ($t(14) = 2.619$, $p_{\text{corr}} = .120$), nor between pre-train and day 2 trained hand ($t(14) = 0.108$, $p_{\text{corr}} = 1.00$), or day 2 untrained hand ($t(14) = 0.614$, $p_{\text{corr}} = 1.00$). There was no difference between post-train PMBR and day 2 trained hand PMBR ($t(14) = -1.083$, $p_{\text{corr}} = 1.00$), or day 2 untrained hand ($t(14) = -1.073$, $p_{\text{corr}} = 1.00$). There was no difference in PMBR between hands

on day 2 ($t(14) = 0.652$, $p_{\text{corr}} = 1.00$) (Figure 5.8C). There were no significant contrasts in the random trials (see Appendix III for results) (Figure 5.8E).

In the 20 Hz group, specifically for the sequence trials, there was a significant decrease in PMBR on the ipsilateral side from pre-train (mean = $4.715\text{E-}15$, $\text{SD} = 2.988\text{E-}15$) to post-train ($1.994\text{E-}15$, $\text{SD} = 1.302\text{E-}15$) ($t(14) = 3.528$, $p_{\text{corr}} = .018$). There was no significant difference between pre-train and day 2 trained hand PMBR ($T(12) = 2.692$, $p_{\text{corr}} = .120$), nor between pre-train and day 2 untrained hand PMBR ($t(11) = 1.735$, $p_{\text{corr}} = .666$). There was no difference between post-train PMBR and day 2 trained hand ($t(12) = -1.508$, $p_{\text{corr}} = .942$), nor day 2 untrained hand ($t(11) = -2.701$, $p_{\text{corr}} = .126$). There was no difference in PMBR between hands on day 2 ($t(11) = -1.560$, $p_{\text{corr}} = .882$) (Figure 5.8D). There were no significant contrasts in the random trials between time points (see Appendix III for results) (Figure 5.8F).

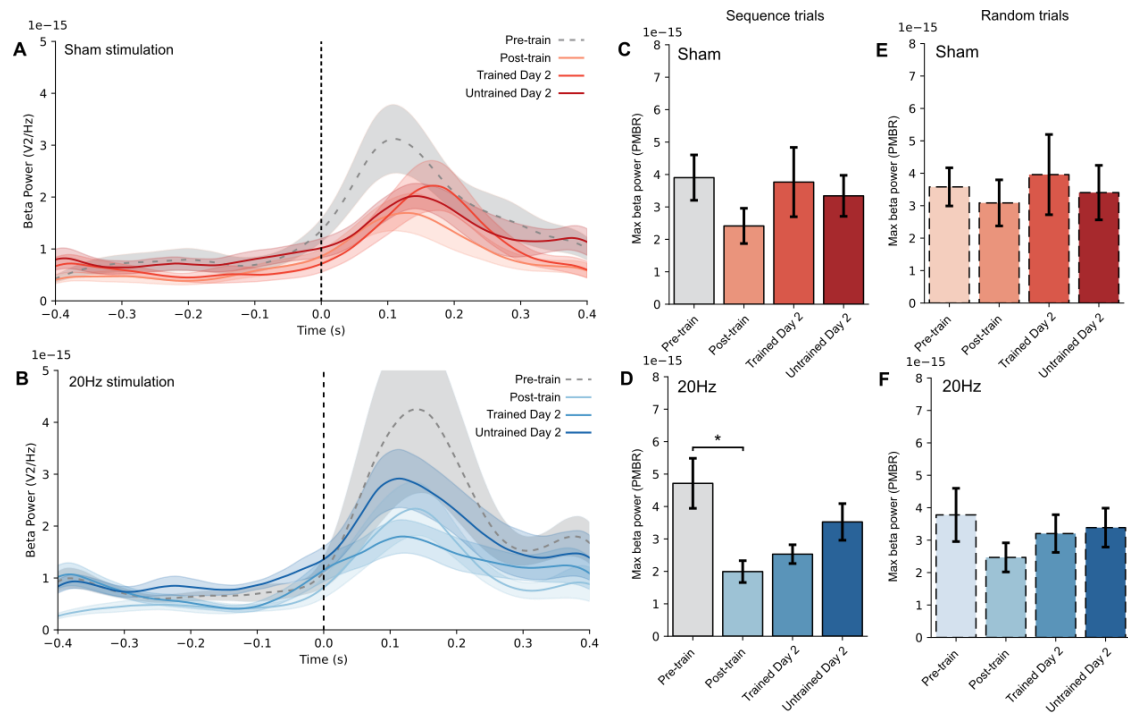


Figure 5.8 Ipsilateral beta activity and PMBR A) Sham stimulation and B) 20 Hz stimulation beta activity during sequence trials over the ipsilateral M1. C) Sham stimulation and D) 20 Hz stimulation PMBR during sequence trials at each time point. E) Sham stimulation and F) 20 Hz stimulation during random trials at each time point. * $p < 0.05$. (Bonferroni corrected).

Given that there was variation between- and within-groups in pre-train PMBR, we then pursued an additional analysis which investigated percent change from baseline PMBR in order to account for baseline differences.

We investigated the effect of trial further by analyzing PMBR in the contralateral and ipsilateral M1 between random and sequence trials at each time point. Paired-sample t-tests were used to compare PMBR between sequence and random conditions at each time point, hemisphere, and within each group. P-values were Bonferroni corrected for number of time-points (3). Where data violated assumptions of normality per Shapiro-Wilk, a Wilcoxon signed-rank test was used instead. In the sham group, there was no significant difference between random and sequence % change in PMBR in the contralateral hemisphere at post-train ($Z = -0.966$, $p_{\text{corr}} = 1.00$), trained day 2 ($t(14) = -0.584$, $p_{\text{corr}} = 1.00$), nor untrained day 2 ($t(14) = -1.280$, $p_{\text{corr}} = 1.00$). There was a significant difference on the ipsilateral side in % change PMBR at the post-train point ($z = -2.556$, $p_{\text{corr}} = .024$), but not during trained day 2 ($t(14) = -1.238$, $p_{\text{corr}} = 1.00$) nor untrained day 2 ($t(14) = -0.503$, $p_{\text{corr}} = 1.00$).

In the 20 Hz group on the contralateral side, there was a significant difference between trials in the post-train PMBR % change ($t(14) = -4.226$, $p_{\text{corr}} = .002$) where the mean % PMBR change of the sequence trials = -37.140, SD = 59.983, and mean % PMBR change of the random trials = 19.226, SD = 80.166). There was a significant difference between trial types in the trained hand ($z = -2.691$, $p_{\text{corr}} = .015$) where the mean % change PMBR of the sequence trials = -45.213, SD = 43.849, and mean % change PMBR of the random trials = 26.203, SD = 113.177. There was no significant difference in the untrained day 2 hand ($t(11) = -1.112$, $p_{\text{corr}} = .387$). There were no difference in % change PMBR between trial types on the ipsilateral side at any of the post-train ($z = -1.306$, $p_{\text{corr}} = .624$), trained day 2 hand ($t(12) = -1.448$, $p_{\text{corr}} = .519$), nor untrained day 2 hand ($t(11) = -1.562$, $p_{\text{corr}} = .438$) (Figure 5.9).

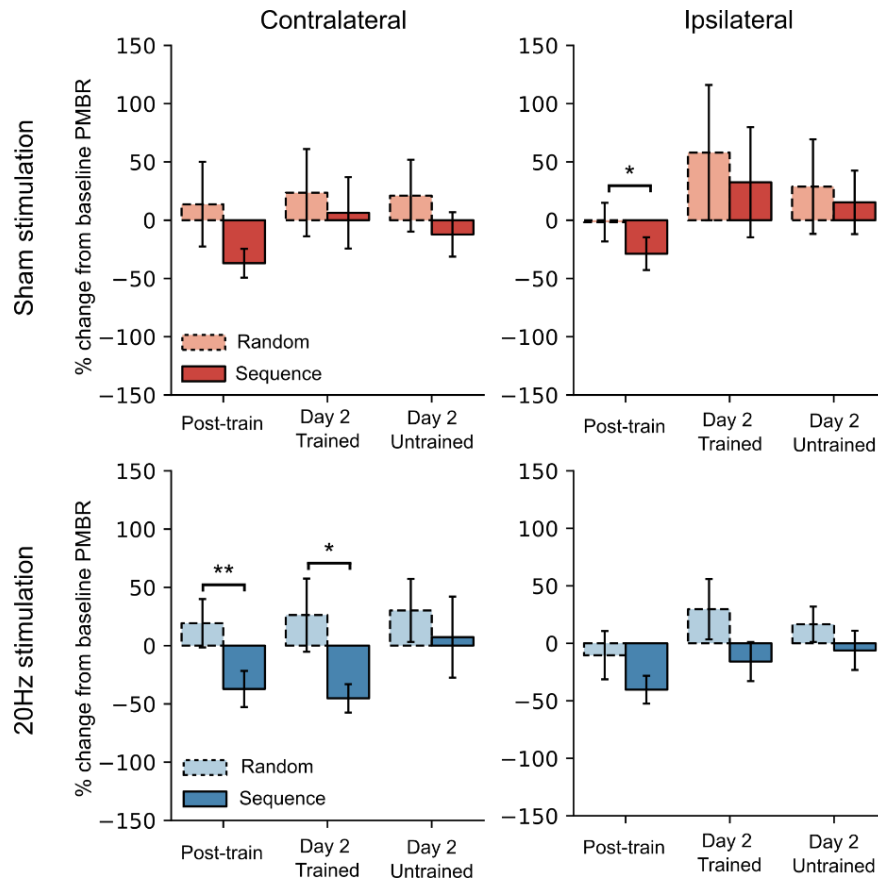


Figure 5.9 % change from baseline PMBR for the sham (upper) and 20 Hz stimulation (lower) on the contralateral (left) and ipsilateral (right) sides during random and sequence trials. * $p < 0.05$, ** $p < 0.01$ (Bonferroni corrected).

In order to relate learning on day 1 with PMBR changes on day 2 (persisting in the 20 Hz group, see Figure 5.7A), we performed Pearson correlations between post-train skill (a measure of learning) and the day 2 PMBR on trained and untrained hand (a baseline-corrected neural correlate reflecting learning). We selected this contrast in order to determine if training-modulated skill would predict correlates of the learning on the next day. Across both groups, there was a significant correlation (Bonferroni ($n=4$) adjusted threshold $p = 0.013$) between post-training skill and contralateral PMBR on the trained hand ($r = -0.523$, $p = .004$, $n = 28$), ipsilateral PMBR on the trained hand ($r = -0.486$, $p = .009$), and contralateral PMBR on the untrained hand ($r = -0.475$, $p = .012$). There was no significant correlation of PMBR on the ipsilateral side of the untrained hand ($r = -0.376$, $p = .053$) (Figure 5.8). These correlations suggest that increased

skill was (in 3/4 of the analyses) correlated with decreases in PMBR on the following day, congruent with the training effects in Figure 5.7.

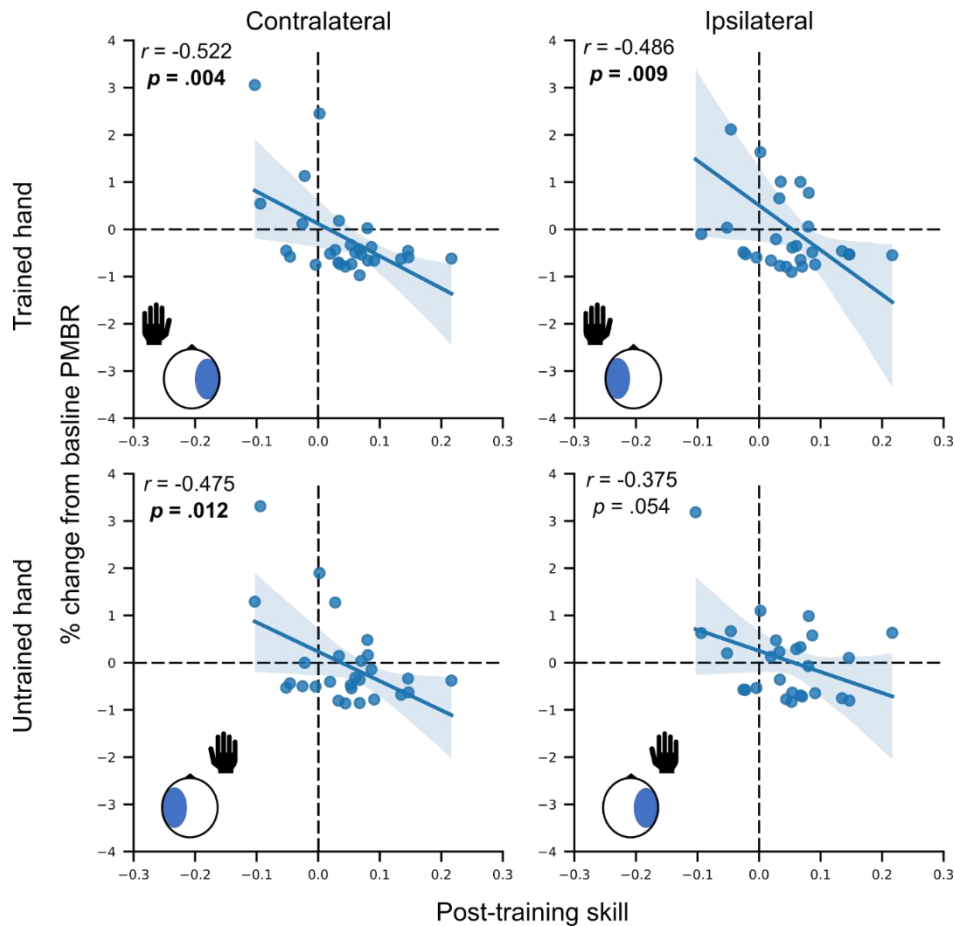


Figure 5.10 Correlation between post-training skill and contralateral (left) and ipsilateral (right) PMBR during trained hand (upper) and untrained hand (lower) testing sessions. Individual data points depict participants, significant correlations are bolded (adjusted for 4 comparisons $p = .013$). 95% confidence interval is indicated by shaded area. Dashed lines indicate 0 skill (vertical) /0% change (horizontal) from baseline.

5.3.6 Supressed iBF after motor learning and stimulation

We analysed contralateral resting-state peak beta-frequency in each group pre- and post-training/stimulation. A GLMM of iBF, with participant added as random factor to account for the individuality of iBF, found a marginally significant interaction between group and hemisphere on iBF ($p = 0.061$), but no main effect of group or side (all $p > 0.05$). Post-hoc contrasts found a

marginally significant difference ($p_{adj} = 0.10$) between the 20 Hz and sham group on the contralateral side, but no significant difference in the ipsilateral side ($p_{adj} = 0.60$) (Figure 5.12).

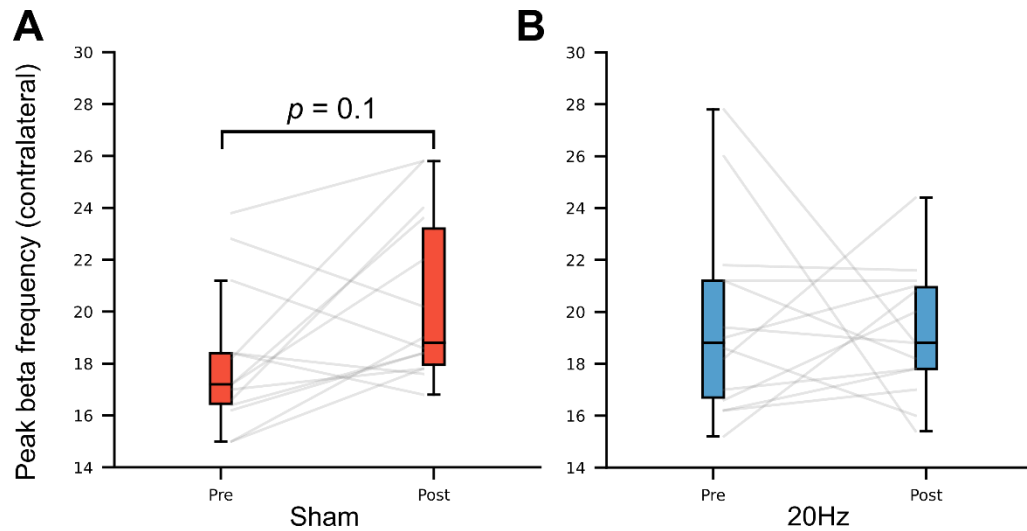


Figure 5.11 A) Resting state iBF in the contralateral motor area pre and post training in the sham group and B) 20 Hz group. Grey lines depict individual change. $*p < 0.05$.

5.3.8 20 Hz stimulation modulates pupil dilation following stimulation offset

We analysed the resting state pupil dilation range in order to determine if there were changes in pupillometry measures from pre- to post-training/stimulation. A GLMM analysis revealed a significant interaction between group and time ($p = 0.010$), and time ($p < 0.001$), and no main effect of group ($p = 0.731$). Post-hoc testing found that there was no significant change from pre to post-training in the sham group ($z = -1.145$, $p_{adj} = 0.252$), however there was a significant decrease from pre to post-training/stimulation in the 20 Hz group ($z = -4.584$, $p_{adj} < 0.001$; pre-training dilation range mean = 7320.982, SD = 398.666, post-training dilation range mean = 6205.473, SD = 337.897) (Figure 5.13).

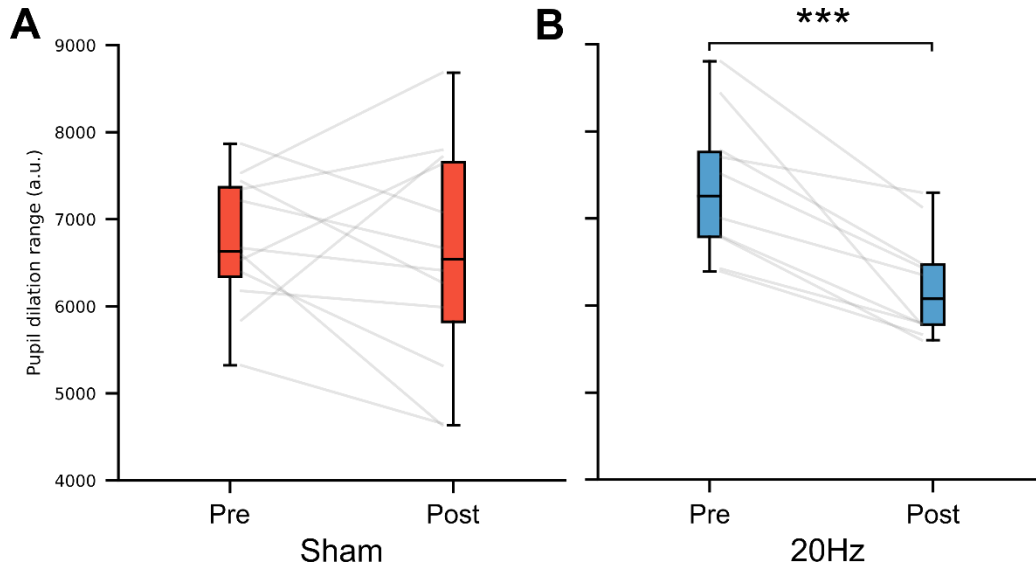


Figure 5.12 A) Pre- and post-train pupil dilation range for the sham group and B) 20 Hz group. Grey lines indicate individual change. * $p < 0.001$.**

5.3.9 No modulation of resting beta power at stimulation sites or M1

In order to determine if there was specific modulation of beta power over either the motor cortices or the electrode sites, we performed two contrasts whereby the pre-post beta power difference at each location of interest was calculated. Over the contralateral M1 (either C1 or C2), there was no significant difference in beta power change between the groups ($t(26) = -0.747, p = .462$) (Figure 5.14B). There were also no group differences in beta power change over the stimulation site ($t(26) = 0.711, p = 0.483$) (Figure 5.14C).

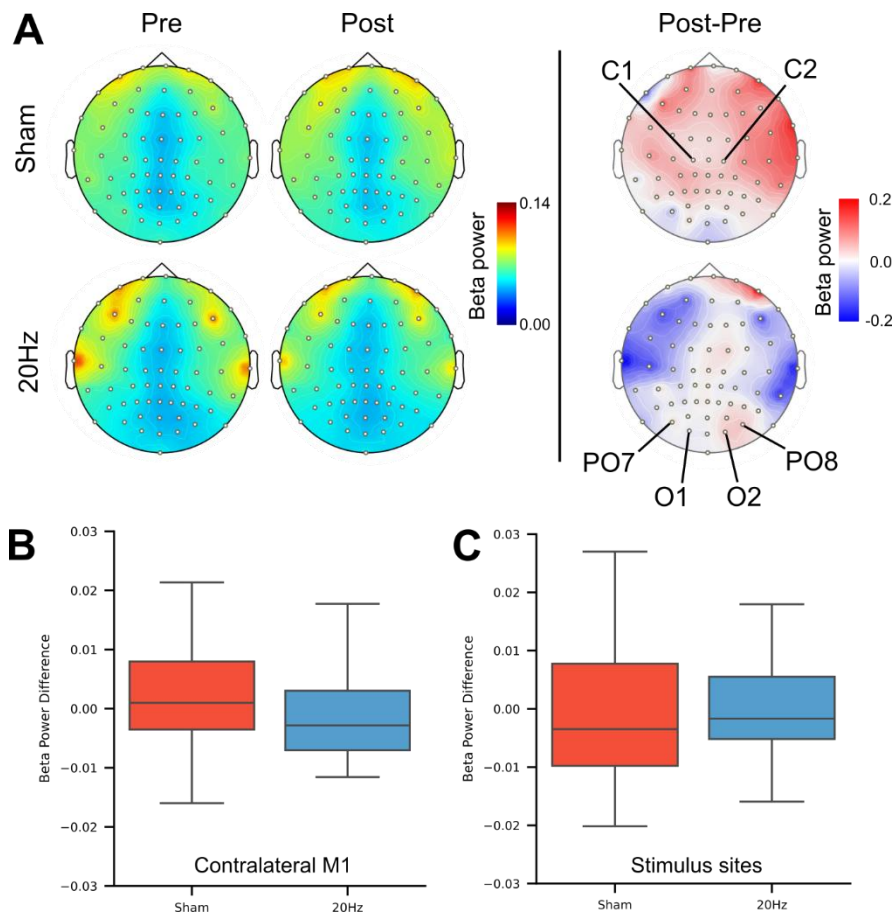


Figure 5.13 A) Beta power (13-29 Hz) topographic plot for sham and 20 Hz groups pre- and post-training. Difference plots (right) indicate average within-subject difference in beta power. Upper difference plot (post-pre) identifies contralateral M1 locations, and lower difference plot indicates sensors underlying stimulation site. **B)** Difference in beta power at contralateral M1 electrode between groups. **C)** Difference in beta power at stimulation site (mean) between groups.

5.4 Discussion

5.4.1 20 Hz stimulation modulates skill acquisition and later transfer

Following the observation in **Chapter 4** that 20 Hz stimulation appeared to modulate motor skill learning, we pursued an extended version of the task with added measurements in order to better understand how 20 Hz stimulation may modulate motor learning compared to sham stimulation. Behaviourally, we noted both immediate and prolonged changes indicating altered motor skill learning in the 20 Hz group compared to the control. Specifically, we note that while the combination of both groups showed significant interference with random trials, only the 20 Hz group had significant difference in reaction time between sequence and random sub-blocks (Training block 2, Table 5.2). There were no significant differences across skill measures, and no significant differences in accuracy, suggesting that reaction time changes due to random trial interference were related to implicit sequence learning and strength of the internal model.

During acquisition, there was no significant difference in overall reaction time as was observed in **Chapter 4**. One explanation could be the introduction of the pre-stimulus baseline block which was intended to identify existing performance differences (we observed no significant differences between groups in the pre-stimulus blocks). In this instance, however, the period of time between baseline and stimulation block(s) which allowed for the experimenter to begin the stimulation and instruct the participant, simultaneously created an extended break resulting in a drastic change in reaction time with no training. Such effect has been documented previously as a rapid form of offline consolidation during breaks (Bönstrup et al., 2019). In the previous chapter, continuous learning with short controlled breaks likely prevented rapid offline learning, while in this instance it could be that the prolonged break resulted in a near-plateau performance rate (visible when comparing the learning rate minimum to that of **Chapter 4**). Thus, while we did not observe the same reaction time changes, interference effects give some insight into the behavioural changes.

On day 2, however, we observed a trend which was not seen in **Chapter 4**. The group receiving sham stimulation had a significant difference in skill between untrained and trained

hands, while the 20 Hz group had no significant difference. Given that both groups would be naïve to performing the task on their dominant hand (and since there were no pre-stimulation differences), we expect that any difference in dominant skill would relate to inter-manual transfer of the learned sequence. Using a transfer index, we confirmed that the 20 Hz group had better hand transfer of skill. Due to the extended learning and longer training period, we reason that consolidatory or prolonged learning effects became more apparent (Robertson et al., 2004; Truong et al., 2023), and are measurable in the inter-manual transfer (Conessa et al., 2023). Accordingly, this is consistent with greater learning/consolidation in the 20 Hz group, but given that stimulation was delivered online, we interpret this difference as being acquisition-related as opposed to the effect of modulated consolidation. There are numerous interpretations of improved inter-manual transfer, however for the purposes of this experiment, we interpret that the degree of difference between the trained and untrained hand indicates how well the skill is transferred (e.g., 100% transfer would equate to equal skill on both hands, and reductions in skill are accordingly interpreted as reduced transfer). Thus, greater transfer ability in the 20 Hz group would indicate that the learned skill was acquired in a way which transferred more effectively. In this experiment, the hand-transfer task was designed such that the finger pressing sequence was mirrored for the untrained hand, resulting in a movement configuration (as opposed to goal configuration) (Cohen et al., 2005). This was also a deliberate decision as it was shown that goal configuration retest develops overnight, while movement-based configurations developed on over the day (Cohen et al., 2005), which allows us to control for sleep-related effects (Conessa et al., 2023; Van den Berg et al., 2019). In the adaptive motor learning framework, we also can consider how the development of the internal model of finger dynamics develops through learning (also discussed in **Chapter 3**). It may be that improved prediction of preceding movement through learning results in a more stable internal model, which is retrievable during hand transfer (especially in a movement configuration). As proposed in **Chapter 4**, NITESGON may indeed alter neural gain and moderate the balance between sensory and predictive input (Aston-Jones & Cohen, 2005a, 2005b), improving learning rates (Jordan, 2024), and creating a more robust internal representation through modulated plasticity (further investigated in **Chapter**

6). This would support the notion of better inter-manual transfer through improved learning. Subsequent findings in this chapter further support these claims neurophysiologically, by considering measures of neural processing, excitability, plasticity, and connectivity.

5.4.2 Resting state features suggest modulated beta activity is congruent with learning

We first considered rsEEG features before and after task learning to understand neural changes which may result from the stimulation and task pairing. We found that post-train interhemispheric beta connectivity correlated negatively with day 2 (retest) skill across both groups. This is primarily consistent with work that showed increased beta connectivity within the motor network was correlated with poorer learning and consolidation (Titone et al., 2022), but more interestingly we found specifically that decreased interhemispheric connectivity resulted in greater *untrained* skill. This puts forth that the ability to transfer or perform the learned sequence in the untrained hand was better in individuals with less correlated interhemispheric activity. This effect also appears to be modulated by 20 Hz stimulation, since it was found that in the 20 Hz stimulation group there was a significant reduction in interhemispheric connectivity, while in the sham group there was no significant change in connectivity from pre- to post-training. One interpretation is that the (contralateral) hemispheric activity during unimanual motor learning is more specific to the learning processes than the ipsilateral, and thus reduced connectivity reflects more distinct hemispheric activity. It may also reflect greater ipsilateral inhibition consistent with better performance in transfer (Birbaumer, 2007; Kobayashi et al., 2009). In either interpretation, the desynchrony in activity between hemispheres has been shown to facilitate improved learning, and we find the same correlation in our data, modulated by 20 Hz stimulation.

We then considered individual beta frequency at rest, given its previous association with endogenous GABA concentrations (Baumgarten et al., 2016). It was reported that greater individual beta frequency correlated with GABA concentration, as a connection between oscillatory activity and molecular concentration. This was also noted in Baumgarten et al. (2016) to be a primarily contralateral phenomenon, and so our investigation looked specifically at

contralateral individual peak beta frequency. We found that the sham group had a significant increase in peak beta frequency, while the 20 Hz group did not have a significant change. If we consider the sham group to be the unmanipulated sample, and naturally the 20 Hz group the modulated sample, then we can interpret the lack of change in the 20 Hz group as a suppression of natural iBF elevation, reflecting reduced GABA concentration post-learning (although to my knowledge no study has investigated iBF dynamics pre- and post-motor sequence learning). Despite this gap, the relationship between GABA and motor learning is an extensively studied area, primarily summarized as a negative relationship, whereby modulation of the GABAergic system via reduction improves motor learning (Stagg et al., 2011). Accordingly, our results could suggest that 20 Hz stimulation modifies GABA and/or beta oscillatory activity, consistent with enhanced plasticity (via GABAergic reduction, and possibly subsequent Glutamatergic elevation) (Eisenstein et al., 2024). Together, these findings illustrate both reduced hemispheric connectivity associated with better motor skill in the untrained hand, and changes in resting peak beta frequency which have been associated with changes in GABA activity. Both of these aspects of resting state activity were modulated in the 20 Hz group, whereby both a decrease in connectivity and a suppression of individual beta frequency change support enhanced motor learning correlates both in terms of representation of connectivity and molecular mechanism. These changes are also consistent with the proposed mechanism in which NITESGON increases activity of the LC, driving changes in neural gain, resulting in altered plasticity and excitability in the motor cortex during learning, as has been demonstrated previously in rodents using VNS (Daniel R Hulse et al., 2016; Porter et al., 2011).

During rest, we also measured pupil dilation as a measure of LC activity, as has been accomplished in similar previous work (Murphy et al., 2014; Seminck et al., 2024; Vanneste et al., 2020). In doing so, we wished to observe if, consistent with the LC stimulation theory, there was detectable evidence of LC-NE system modulation. Furthermore, numerous neurotransmitter systems including cholinergic and adrenergic systems have been associated with pupil size changes (Reimer et al., 2016). Thus, recording pupil dilation at rest following stimulation would

allow us to gauge the effect of stimulation on either of these systems, and points to a possible mechanism for NITESGON. Interestingly, and inverse to the expected outcome, we found a significant reduction in pupil size in the 20 Hz group, compared to no significant change in the sham group. Although counterintuitive, we put forth that such a consistent trend (all individuals receiving 20 Hz stimulation had reductions in pupil size), is reflective of modulated LC-NE activity. One interpretation could be that given the timing of recording (resting state *after* the stimulation), that modulated LC activity resulting in the expected pupil dilation had subsided or indeed compensated, resulting in a compensatory decrease in LC activity and parasympathetic activation leading to pupil constriction. We note however that this has yet to be investigated in humans.

5.4.3 Neural responses are consistent with adaptive motor learning and changes in cortical gain

Despite the evidence in the rsEEG results would highlight group differences interpreted as improved motor learning correlates, it remains difficult to uncover the mechanism by which 20 Hz NITESGON might influence these aspects, and indeed whether there is a casual link between the correlated measures presented above. I earlier introduced framework around ERPs associated with motor learning, specifically the PMBR, which has been associated with GABAergic pathways, processing time, and error (Heinrichs-Graham et al., 2017; Tan et al., 2014; Zhang et al., 2024). Our results found that, contralaterally, the sham group showed a significant decrease in sequence-related PMBR amplitude from pre-train to post-train blocks, but no significant difference from pre-train amplitude in the successive trained and untrained hand retest on day 2. There was also no difference in random trial PMBR across blocks, suggesting that reductions in PMBR are learning-related. Conversely, the 20 Hz group also showed a significant reduction in PMBR amplitude following training, and this reduction also carried into the day 2 trained hand blocks. Similar to the sham group, the random trial PMBR amplitude did not differ across training blocks, further supporting the notion that PMBR reduction is learning-related. The random vs sequence PMBR change was further explored using a baselined measure to account for existing individual and group differences, and similarly found that while there was

no contralateral differences in % PMBR change in the sham group, the 20 Hz group had a significant difference between random and sequence % PMBR change. This aspect was further supported behaviourally, as we found that post-training skill correlated negatively with (baseline corrected) PMBR reductions on the next day. This relationship suggests that individuals with greater skill during day 2 testing had the greater reductions in PMBR (a relationship significant in both hemispheres for the trained hand, but only the contralateral hemisphere in the untrained hand).

These findings would be consistent with the PMBR reflecting balancing between prediction and sensory input, as part of the framework surrounding neural gain via computed prediction error (Jordan, 2024). In this theory, neural responses are proposed to be larger when dependent on sensory input, and through learning are attenuated as predictive internal model inputs take over (Figure 5.18A). LC stimulation is proposed to modulate this balance, which also moderates plasticity, whereby incoming sensory input drives plasticity (through learning), and prediction-driven performance inhibits plasticity where learning is unnecessary (where a stable internal model is available).

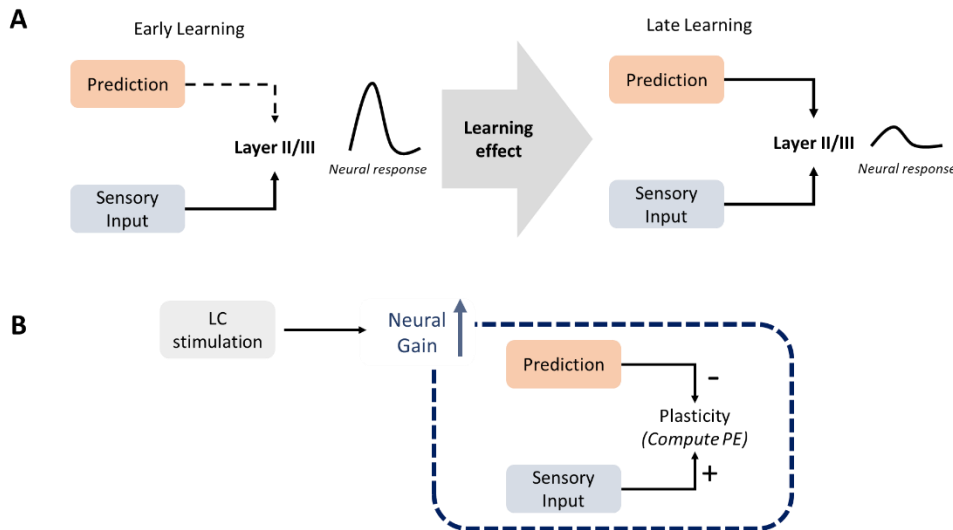


Figure 5.14 Neural gain driven by LC activity and its influence on weighted prediction vs sensory input. A) Early learning and late learning input from model (prediction) and external (sensory) information sources. Early learning corresponds to a greater neural response with more reliance on sensory input (compounded by error correction and model updating). Late learning is characterized by a reduction in reliance on sensory input and greater input by the internal model (prediction), corresponding to reduced neural responses. B) LC driven neural gain changes mediating the balance of (A). Figure based on Jordan (2024).

While this interpretation fits with the current model and proposed theory, it also contradicts numerous other investigations of the PMBR whereby it was shown that reductions in PMBR amplitude reflect error processing and uncertainty (with subsequent updating of the internal model) (Cao & Hu, 2016; Korka et al., 2023), and increased PMBR reflects confidence, or the maintenance of the existing internal model (Cao & Hu, 2016). While there is strong theoretical and experimental evidence that these interpretations are correct, our findings provide the inverse, particularly when compared a decreasing PMBR in learnable trials to an unchanging one in the unlearnable trials. I then put forth that cognitive demands and task difficulty are important aspects in this paradigm, and may explain the seemingly contradictory results. In the current PMBR theory, error processing results in decreased PMBR amplitude and updating of the internal model. Thus we would expect that through learning, PMBR should increase reflecting an increasing reliance on the internal model and less updating (due to less errors). The task here however has a high (and constant) accuracy rate, which has previously been shown to influence learning and performance (Kennedy et al., 2021), and which also suggests that ‘model updating’

occurs less frequently through error. Accordingly, we would not expect error-driven model updating as the driver of PMBR modulation, especially given the constant accuracy. PMBR could instead reflect reliance on the external sensory vs the internal model input (effectively represented in random vs sequence trials). This is supported by an unchanging PMBR during random trials, which would ‘always’ rely on sensory input given its unpredictability, and a decreasing PMBR during sequence trials which would reflect a growing reliance on an internal model which was developed through learning, as has been proposed previously (Arnal & Giraud, 2012). It may also be that in predicting the successive button press, PMBR is reduced to allow for faster initiation of the subsequent action (a phenomenon which has been shown previously, and which was attributed to cognitive strategy and preparation (Korka et al., 2023)). Conclusively then, reduced PMBR appears to represent improved prediction (either via strategy or enhanced model dependence), and 20 Hz stimulation resulted in prolonged PMBR reductions not observed in the sham group, potentially indicating a more stable or recoverable internal model. This is consistent with an LC-implicating theory, given that reliance on internal model vs external sensory input is mediated via cortical gain controlled by the LC (Jordan, 2024). As an interesting addition, we observed a behavioural effect in accuracy consistent with this theory. We reported that when sub-blocks changed from sequence to random trials, an interference designed to observe slowed reaction times and gauge motor learning, that the sham group had greater reductions in accuracy (but no change in reaction time). When taken from the predictive model perspective, this decrease in accuracy could be consistent with a continued reliance on the (now incorrect) internal model, as opposed to effective switching to sensory input required during random unpredictable trials. Conversely, the lack of accuracy reduction in the 20 Hz group could indicate more effective switching from the internal model to the external sensory input, once it was detected that the internal model was no longer effectively predicting the subsequent button press. This proposal would be highly consistent with the LC as a global model failure system, and indeed would reflect behavioural modification aligned with greater LC output in the stimulation group (see Figure 3 in Jordan (2024)).

5.4.5 Little evidence for transcranial mechanism

In order to further test the theory that the group effects presented above are driven via the LC, particularly in the context of the Lidocaine group in **Chapter 4**, we pursued an EEG power analysis in two ROIs to compare entrainment or power modulation (Otero et al., 2020). In previous studies, tACS produced modulated neural activity with after-effects measurable with EEG (Fang et al., 2024; Kasten et al., 2016). We put forth that if NITESGON elicited its effects transcranially, that after-effects could be detectable in modulated beta power via entrainment. We pursued ROIs in both the motor cortex (central) and the stimulation sites (occipital) to determine if the sham and 20 Hz groups differed. We found that neither analysis revealed altered beta power difference between groups (nor indeed, beta power modulation at all). This would then suggest that 20 Hz NITESGON does not produce measurable changes in beta oscillations following stimulation, and thus no support for transcranial mechanism. This also posits that the behavioural and electrophysiological changes observed following stimulation were caused by a separate mechanism. This also adds to growing literature which reports standard intensity of tACS does not alter neural activity as previously proposed (Ibáñez et al., 2023; Miles Wischniewski et al., 2019). It should be noted, however, that outside of direct tES, post-training entrainment has also not been observed while during-stimulation entrainment was measurable (Seminck et al., 2024), and thus it is not conclusive based on our data to state that entrainment is not a mechanistic explanation for NITESGON's effects.

5.4.6 Limitations and further work

There are several limitations of this chapter which we wish to highlight. As previously identified, we were unable to replicate the significant changes overall task acquisition (reaction time) as was found in **Chapter 4**. This is likely due to several reasons including the addition of a pre-stimulation block which may already acquaint participants with the sequence and task, and dilute modulated behavioural differences. We also noted that extensive break times not allowed during **Chapter 4** created drops in reaction times during periods of rest, which may also reduce

the ‘modulatable’ learning aspects. Despite this, we did observe changes in skill, and so behavioural data still supported improvement.

Our other limitation concerns the lack of physiological recording during the task and stimulation. While we recorded EEG data during the task and stimulation, saturation of the reference and regional electrodes around the stimulation site prevented robust analysis of the EEG signal. Furthermore, there is ongoing debate surrounding whether tACS artifact can be effectively removed from E/MEG signals, especially when investigating the frequency which is also the stimulating frequency (for an extensive discussion, see the original paper by Noury et al. (2016), the rebuttal by Neuling et al. (2017), and further reply by Noury and Siegel (2018)). Thus, we opted not to pursue analysis of the simultaneous tACS-EEG data. We also did not record pupillometry during the task, an aspect which obscures the validity of the findings in section 5.3.8. While we interpreted the stark reduction of pupil diameter following stimulation as an indicator of LC-NE involvement, its surprising direction would be better validated with simultaneous recording with tACS. We note that NITESGON has previously been carried out with pupillometry which did find increased pupil dilation consistent with increased LC output and noradrenergic activity (Vanneste et al., 2020), but we were not able to conclude the same in this study.

Given that we observed unexpected pupillometry results, and indeed found the effect of beta stimulation to be more efficacious over low-gamma range, we put forth that as an alternative explanation for the observed changes, it is possible that the effects are driven by entrainment via peripheral activity to the motor cortex. Such entrainment has been reported before (Asamoah et al., 2019), and would indeed be a parsimonious explanation for the frequency-specific effects observed. It may also indeed explain why differences in pupillometry were inconsistent with increases in central NA.

The next steps of this research will be to observe additional measures of physiological modulation which support the LC-NE involvement more directly (e.g., 7T MRI recording of LC activity during stimulation (Morris et al., 2020), measurement of neural plasticity (e.g., TMS +

tACS (Guerra et al., 2019), and finally to determine if NITESGON can be integrated into clinical settings for motor dysfunction such as stroke recovery (Yang et al., 2023). Accordingly, in **Chapter 7**, we pursue a final experiment using TMS-measured motor cortex excitability to investigate changes in neural plasticity consistent with improved motor learning.

5.5 Conclusion

In conclusion, in **Chapter 5** we showed behavioural modulation consistent with improved motor learning and inter-manual transfer in the 20 Hz. We show that 20 Hz stimulation modulates rsEEG connectivity, correlated with improved motor learning, and individual peak beta frequency, consistent with GABAergic activity. We also found reduced neural responses during learned vs unlearned trials, and modulation of this reduction in the 20 Hz group. Overall, our results support that 20 Hz stimulation improves motor learning behaviour and electrophysiological correlates, consistent with the adaptive motor learning and LC as a global model failure system theories, with no evidence of a transcranial mechanism.

Chapter 6 Preliminary Evidence of the Effect of Peripheral Nerve Stimulation on Neural Plasticity

6.1 Introduction

Following the successful behavioural modulation and subsequent identification of electrophysiological and physiological correlates of motor learning modulation in the previous chapters, the final experiment of this thesis intended to measure an additional physiological correlate of motor learning and neural plasticity through cortical excitability. Cortical excitability has been previously measured with good success using transcranial magnetic stimulation (TMS), a method which delivers pulses of magnetic field which induces an electrical current in the targeted tissue (Bestmann & Krakauer, 2015). When delivered to the motor cortex, specifically in regions corresponding to hand control (Kim et al., 2023), this pulse can evoke muscle responses measured as a motor evoked potential (MEP). The MEP represents both cortical and spinal activity, and indeed the individual components contributing to its amplitude are complex and difficult to detangle using single-pulse TMS. Despite this, they are still often used to measure excitability of the cortico-spinal system (Bestmann & Krakauer, 2015). Among numerous applications, it has been shown that the acquisition of motor skill through learning produces reductions in TMS threshold, and increases in motor map representation (Cohen et al., 1998), and this observation appears age-independent (Cirillo et al., 2011). In this experiment, we aim to further the understanding of the effects of NITESGON paired with motor learning by probing motor cortex plasticity changes between an active and sham stimulation group. Alterations in cortical excitability as a result of noradrenaline have already been successfully measured in previous work (H. I. Kuo et al., 2017), whereby administration of noradrenaline reuptake inhibitors produced increased cortical excitability. Following the proposal in this thesis, and consistent with previous work which states that NITESGON appears to act on the LC-NE system to drive plasticity and modulation, we reason that TMS MEPs could be useful to gauge the effect of NITESGON on motor cortex plasticity.

Previous work pairing TMS and tACS has found that 50 Hz tACS to the cerebellum produced MEP increases and better adaptation in a motor sequence task (Naro et al., 2016). Similarly, 40 Hz tACS delivered over M1 resulted in reductions in speed during a motor tapping task, and subsequent reductions in cortical reactivity measured through MEP amplitude (Giustiniani et al., 2019a). Thus, there is evidence that TMS can be used to measure both increases and decreases in cortical excitability induced by tACS. Therefore, in the final experiment of this thesis we carried out a replication experiment of **Chapter 5**, with added TMS measurement at several time points and across both hemispheres in order to measure changes in excitability and cortical plasticity.

6.2 Methods

6.2.1 Participants

Since data collection was carried out at the Brai3n Clinic in Ghent, Belgium, translated versions of the BDI, BAI, handedness inventory, and task version were used. These translated copies are available in Appendix IV. Practical limitations and patient exclusion resulted in a smaller sample size than the previous chapters, and so the work here is presented as preliminary evidence.

6.2.2 Experimental procedure

*The experimental procedure follows an identical task and paradigm as in **Chapter 5**, however without EEG and pupillometry, and with the addition of recording TMS MEPs. The procedure is reproduced here in brief for clarity.*

Participants were asked to complete a version of the serial reaction time task (SRTT) while receiving stimulation, and return for a second session on the following day. Participants were not informed of which group they were assigned, but were told that there were both active and control conditions, and both produce similar sensations due to the blinding procedure. Following the 15-minute SRTT, participants were asked to return 24 hours later to complete a

shortened version of the task. Participants used their non-dominant hand during the training (day 1) session.

The training day 1 SRTT followed a typical motor learning design and included the presentation of a visual stimulus in the form of 4 vertical rectangles (Figure 4.1) on a 60cm × 34cm monitor with 75 Hz refresh rate and 1024×768 resolution. Participants sat approximately 60 cm from the screen, and placed their 2nd through 5th digits (pointer finger to little finger) on the H, J, K, and L keys on a keyboard. (If non-dominant was left, little finger was on H, and if non-dominant was right, pointer finger was on H). Participants were asked to press the button on the keyboard corresponding to the location of the visual stimulus (rectangle darkened). Participants were not told of the existence of any sequence and were simply instructed to respond as quickly and accurately as possible, with no preference for either. After 24 hours, participants returned to perform a shortened version of the task without stimulation and without any auditory stimulus to better gauge the extent of motor learning without a secondary task. Participants first completed one grouping of R-S-R on their non-dominant hand, and after a 30 second break, were asked to complete the remaining blocks on their dominant hand. Before and after day 1 training and day 2 testing, participant MEPs were recorded (Figure 6.1).

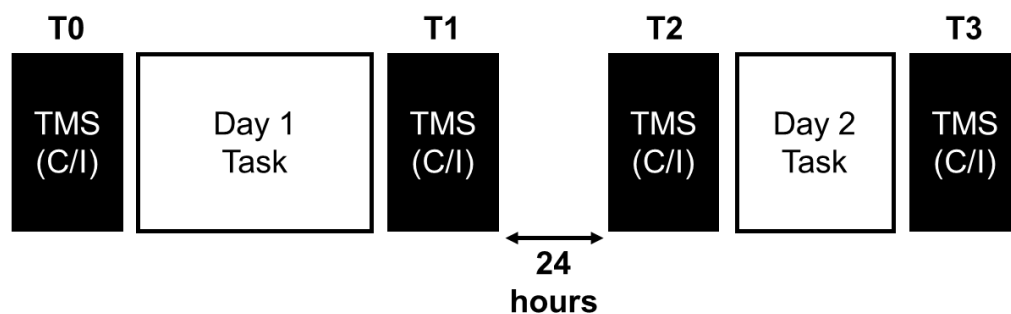


Figure 6.1 TMS + NITESGON paradigm and delivery timing. 25 contralateral (C) and ipsilateral (I) MEPs are collected at each timepoint (T0-T4).

6.2.3 Stimulation protocol

Stimulation was delivered to the C2 dermatome using a DC-Stimulation Plus device (NeuroConn, Germany). Saline soaked electrodes (35cm²) were placed with anode on the left side of the dermatome, and cathode on the right, wires facing inward (Figure 4.3). This placement follows previous work which identified the occipital nerve as a target (Luckey, McLeod, Huang, et al., 2022; Luckey, McLeod, Mohan, & Vanneste, 2022; Vanneste et al., 2020). During the 1 Hz stimulation condition, stimulation delivered in a sinusoidal wave was ramped up over 30 seconds to 1.5mA at 1 Hz frequency, which lasted the duration of the task, followed by a 30 second ramp-down, before the participant was told to begin the task. For the active condition (20 Hz), stimulation was delivered for the duration of the training session (~25 minutes, 5 mins × 5 blocks) at 1.5mA, with a ramp-up and ramp-down of 2 seconds. Participants were instructed to begin after 30 seconds to allow time for complete ramp-up.

6.2.4 TMS protocol

In order to measure MEPs and how NITESGON may alter motor cortex excitability, we delivered TMS pulses before and after each training and testing session. TMS data was collected using a Neurosoft magnetic stimulator (Neuro-MS/D therapeutic Neurosoft, Russia) with figure-eight coil, in-line with the recommendations for safe collection by Rossi et al. (2021). Participants were seated comfortably in a chair, with neck secured with a pillow and towel. Participants were asked to relax their arms and rest with their palms facing upwards. On both hands, electromyography (EMG) recording electrodes were placed in on the main section of the first dorsal interosseous muscles (active electrode), the proximal interphalangeal joint (reference) and ulnar styloid processes (grounding). Beginning with the non-dominant hand (contralateral hemisphere), TMS pulses were delivered with coil held tangent to the scalp with handle pointing caudally at 45° to the midline to allow current to flow posterior–anterior. Pulses were first tested at 65% of stimulus output to identify the motor hotspot. Once identified, stimulus output was adjusted until the MEP reached 1mV response peak-to-peak amplitude (STIM_{1mV}). The procedure was repeated in both hemispheres. A baseline of 25 monophasic pulses (Cavaleri et al., 2017)

were delivered before the task to both hemispheres, of which MEP amplitude and latency are recorded. Following the task, participants repeated the procedure. On day 2, participants again repeated the same procedure, with 25 pulses delivered at $STIM_{1mV}$ to each hemisphere before and after the testing.

6.2.5 Analysis

Behavioural data was analyzed in the same manner as data in **Chapter 4** and **5**. This includes preprocessing steps to remove data greater than 3 standard deviations above the mean or less than 200ms, and GLMM analysis of reaction time and accuracy data. Sensation data were compared between groups using t-tests. Skill was calculated following *Equation 4.1*, and inter-manual transfer index using *Equation 4.2*.

TMS data was automatedly preprocessed during collection. Thus, where MEPs which could not be detected due to minimal amplitude, data was not recorded. Some participants therefore do not have data for the total (25) number of pulses since only 25 pulses were delivered per person regardless of data viability, in order to maintain consistency between individuals.

Generalized linear mixed models with Gaussian log link used to investigate the effects of time-point, hemisphere, and group on MEP amplitude and latency. This was largely selected so as to account for missing data which can occur during the TMS acquisition (automatedly). Thus, the dependent variable was the MEP amplitude, fixed effects were the group and time-point (pre and post-train), and random effects was subject ID.

6.3 Results

6.3.1 20 Hz stimulation modulates contralateral cortical MEP on day 1

We used a GLMM with effects of group and time to investigate MEP modulation individually for the contralateral and ipsilateral hemispheres. Contralateral, both group ($p = 0.017$), time ($p = 0.002$) and the interaction of group and time ($p = 0.026$) were significant. There was no existing difference prior to stimulation between group MEPs ($p_{adj} = 0.277$), however

following training the 20 Hz group had significantly greater MEP than the 1 Hz group ($p_{\text{adj}} = 0.019$) (Figure 6.2Ac5). Uniquely, the 1 Hz stimulation group had no significant change in MEP following training ($p_{\text{adj}} = 0.649$), while the 20 Hz group had a significant increase ($p_{\text{adj}} < 0.001$). Ipsilaterally, the same GLMM found a weak main effect of Group ($p = 0.064$), and no significant effect of time ($p = 0.968$) nor interaction between them ($p = 0.463$).

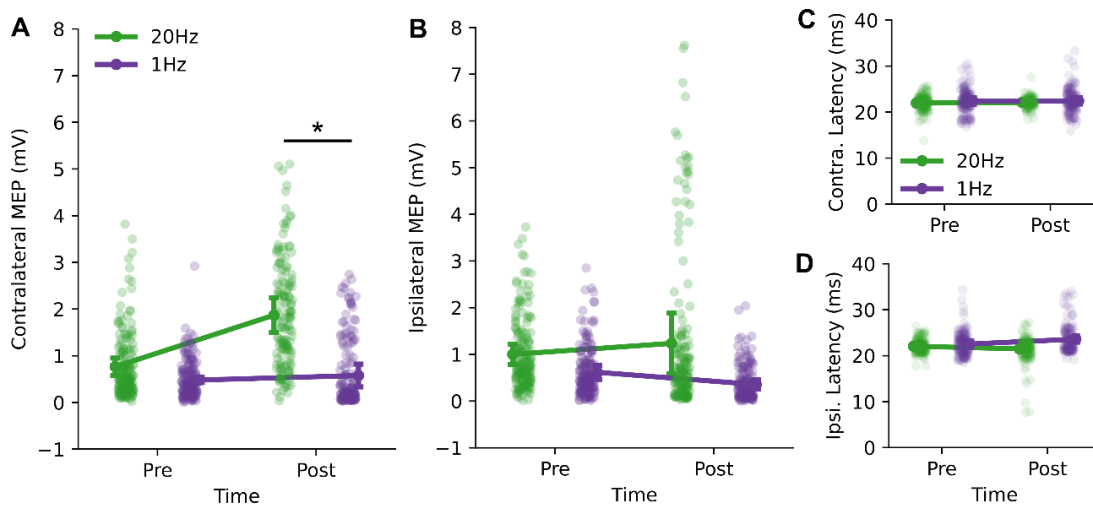


Figure 6.2 (A) MEPs evoked during day 1 training at pre (T0) and post-train (T1) time points. (B) Latency of MEP evoked during T0 and T1 time points. Error bars denote SEM. * $p < 0.05$.

6.3.2 No persisting MEP modulation 24 hours after stimulation

TMS MEPs were measured 24 hours following initial collection on day 1 in the same manner. Data were similarly analyzed using a GLMM on both MEP amplitude between groups at each time point (pre and post-testing), and latency, both for contralateral and ipsilateral sides. On the contralateral side (Figure 6.3A), there was no main effect of group, time, nor their interaction (all $p > 0.05$). Similarly, the ipsilateral side showed no significant effects nor interactions (Figure 6.3B). Following testing, MEPs were measured again, in which there were no significant main effects of group or time, nor their interaction on either the contralateral or ipsilateral side. All latency measures were also non-significant in effect and interaction.

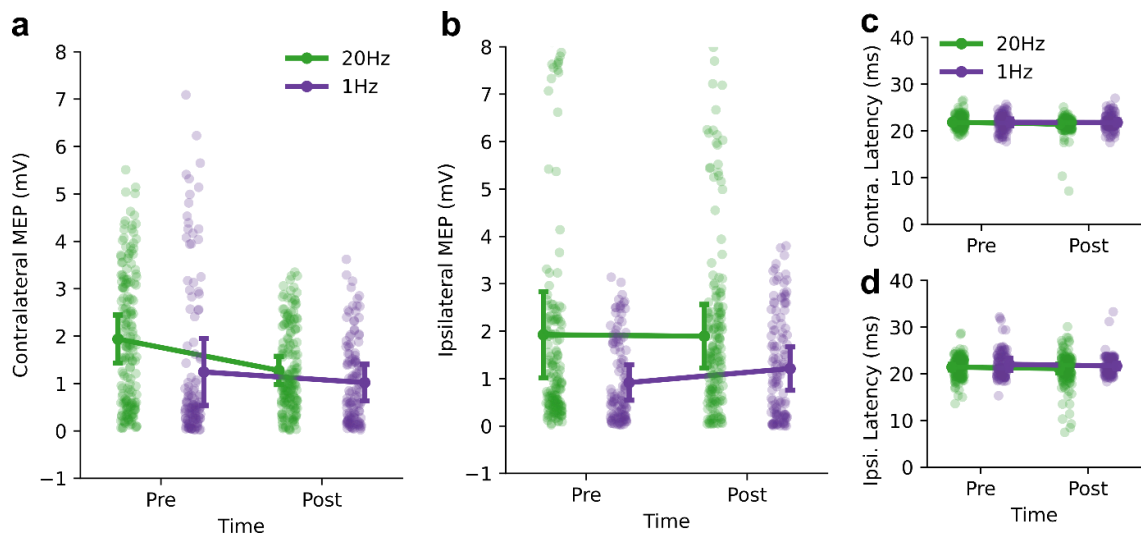


Figure 6.3 (A) MEPs evoked during day 2 testing at pre (T0) and post-train (T1) time points. (B) Latency of MEP evoked during T0 and T1 time points. Error bars denote SEM. * $p < 0.05$.

6.3.4 Behavioural results

The behavioural data within this sample was analyzed to investigate differences in reaction time, accuracy, and skill during day 1 training and day 2 testing sessions. Given the sample size ($n=7/\text{group}$) in comparison to our original samples in **Chapter 4** and **Chapter 5**, we were under power to reliably detect behavioural differences. Specifically, we found that relative skill differences between the 20 Hz and 1 Hz group in **Chapter 4** were significant with a power 0.405 (Cohen's d). A power analysis with a minimal effect size of 0.405, type I error rate of 0.05, and sample size per group of $n = 5$ ($N1/N2 = 1$) calculated that the study would have a power of 0.145. Thus behavioural data at this sample size are not reliable to detect differences. The demographic are presented in the Appendix IV for reference.

6.4 Discussion

6.4.1. 20 Hz stimulation modulates cortical plasticity in the engaged hemisphere

Here, we report preliminary evidence that NITESGON paired with a motor learning task produces modulated MEP amplitude at 20 Hz compared to 1 Hz stimulation. A GLMM analysis revealed a significant interaction between stimulation group and timepoint (pre and post

stimulation), whereby the 20 Hz stimulation group showed heightened MEP amplitude following stimulation and task. TACS-induced MEP modulation has been reported previously (Fresnoza et al., 2018; Giustiniani et al., 2019b), and worsening behaviour and reductions in cortical excitability measured by TMS were interpreted as a weakening of network oscillations and subsequent reduction in synaptic strength in said networks. We offer a different interpretation here, considering that in the previous chapters we identified a peripheral mechanism via blocking of the nerve using lidocaine, and failed to identify lasting neural entrainment in the motor cortex which would otherwise explain changes in cortical excitability. Instead, the implication of the LC-NE system could explain changes in cortical excitability measured by MEP amplitude increases. There is direct experimental evidence that pharmacologically increased NE via reuptake inhibitors resulted in greater cortical excitability (measured via TMS) (H. I. Kuo et al., 2017), and combining norepinephrine reuptake inhibition with a motor learning task also enhanced motor skill acquisition, with corticomotor excitability increases measured via TMS (Plewnia et al., 2004). If we consider that NITESGON likely modulates neural activity via altered NE activity, similar to how the effects of VNS in rodents (Hulsey et al., 2019; Tseng et al., 2021) and humans (Jigo et al., 2024) implicate NE (or generally sympathetic) activity, then modulated MEP amplitude could reflect NA-driven cortical plasticity, identifying a potential mechanism for NITESGON to elicit its behavioural effects.

6.4.2 Plasticity effects are temporally constrained

Our results also revealed no significant change in MEP on day 2 from before to after re-test on the task. We opted not to perform an analysis on the day 2 pre-task MEPs alone, since we could not reliably determine if differences resulted from overnight maintenance of increased excitability, or if they were instead a methodological artifact arising from altered coil positioning. Such artifact would be alleviated with neuronavigation, a method in which computer-based tracking can be used to co-register the patient's head and TMS coil for reliable repositioning (Caulfield et al., 2022), and so we recognize this as a practical constraint in this study, which is further discussed in the limitations. Our original expectation in carrying out TMS measurements

during the day 2 collection was to identify if re-testing on the task would re-induce cortical plasticity, indicating extended learning via processes such as use-dependent plasticity (UDP) (Mawase et al., 2017). A lack of difference between groups, or indeed any significant change in MEPs or latency across the groups (no main effect of time), indicates that the short and inter-manual demands of the task were insufficient to produce measurable differences in MEPs at this sample size. We therefore consider that since measurable differences in MEPs were clear following the stimulation and motor task, and modulated by 20 Hz stimulation specifically, that there is a temporal constraint on these effects, which has also been established in previous work, where repetitive paired-pulse TMS increased MEPs for between 10-15 minutes following stimulation (Hamada et al., 2007; Nakazono et al., 2021). Given that we also reported no reaction time or skill differences between groups on day 2 in **Chapter 4** and **5**, it appears that modulation of motor learning is concurrent with the stimulation, and that the after-effect on EEG correlates and MEPs is restricted to a period of time following the stimulation, but not into day 2 re-testing.

6.4.3 Limitations

The primary limitation of this preliminary work is the sample size, which although was sufficient to identify significant effects of 20 Hz NITESGON on MEPs, still falls below standard sizes for definitive studies of MEPs (Pagge et al., 2024). This also prevented meaningful results from the behavioural aspect of the study, which was believe was prone to age-drive variability (Fresnoza et al., 2020; Fresnoza et al., 2018), which was a unique feature of the population in this study compared to the previous studies. While age has generally not been considered to be an influential factor for the implicit SRTT, as ours was, we consider that LC integrity which has been shown to change through aging (Manaye et al., 1995), might introduce effect variability in the studied population. This aspect raises important considerations for translation of NITESGON into clinical groups primarily composed of older populations, since treatment efficacy may be dependent on structural and functional integrity of the engaged system, namely the regions of the LC-NE system including nerve health, LC integrity, and overall white and grey matter volume (Cobia et al., 2024; Wang et al., 2014).

The addition of TMS in this experiment also opens possibilities for future investigations of the mechanism of NITESGON in this context. While the current equipment in this experiment did not permit more temporally precise investigations, coupling tES and TMS pulses could offer a unique opportunity to understand if phase of the stimulation has an entraining effect on the motor cortex, or if plasticity is instead indirectly driven by subcortical structures (i.e., the LC). Future research investigating peripheral nerve stimulation may seek to utilize TMS and tES coupling to further elucidate the mechanism of occipital nerve stimulation.

6.5 Conclusion

In conclusion, in **Chapter 6**, we showed that 20 Hz NITESGON delivered concurrent with a motor learning task produced increased MEP amplitudes consistent with increased motor cortex excitability. 1 Hz stimulation did not produce this effect, suggesting that cortical excitability and neural plasticity are modulated by 20 Hz stimulation of the occipital nerve during a motor learning task. Effects were constrained to day 1 training, as they were not observed following re-test in the following day. These results suggest that altered neural plasticity may explain the behavioural improvements in the previous chapters, and further implicate the role of the LC-NE system.

Chapter 7 General Discussion

7.1 Summary

Many years of research looking at how tACS can modulate motor learning have produced promising, although often inconsistent results (Bland, 2021; Ibáñez et al., 2023). The prospect of modulating motor learning for both practical and clinical applications had thus supported alternative mechanisms and hypotheses, such as the stimulation of peripheral nerves as a means of modulating behaviour (Asamoah et al., 2019). While there has been fundamental advancement in the pre-translational evidence for peripheral nerve stimulation and motor control/recovery (Buell et al., 2018; Hays et al., 2016; Hays et al., 2015; Daniel R. Hulsey et al., 2016; Hulsey et al., 2017; Hulsey et al., 2019; Khodaparast et al., 2013; Khodaparast et al., 2016; Tseng et al., 2021), there has only been initial evidence of the same in humans (Asamoah et al., 2019; Seminck et al., 2024). Taking these aspects into account, I carried out a series of works which together test if peripheral nerve stimulation in humans can modulate motor learning, how stimulation can alter neural activity, and whether these changes relate to cortical excitability. I discuss how these findings fit with current adaptative motor learning framework to identify a mechanism, positing future work using this method. To that end, and relating to the original hypotheses proposed at the onset of this thesis, I identified the theoretical basis for a peripheral mechanism as it relates to the current transcranial approaches used in motor research. This included reconsidering the mechanism and effects of tACS/tDCS through the peripheral nerves. Next, I performed a meta-analysis of motor learning studies to identify fMRI correlates of motor learning processes both online and offline. The results of the analysis were consistent with current adaptive motor learning framework, and supported online targeting for tACS stimulation to alter neural activity relating to these processes. In the first empirical experiment (**Chapter 4**), I present the first experimental evidence in humans that occipital nerve stimulation modulates motor learning with frequency specificity. This was confirmed using behavioural markers of motor learning, and a secondary experiment using topical anaesthesia identified that the mechanism of NITESGON-

modulated skill acquisition in this context appears primarily transcutaneous. In the second experiment (**Chapter 5**), I identified neural correlates of motor learning using event-related and resting state EEG. These findings were consistent with the adaptive motor learning framework, and were also consistent with a model in which LC activity drives the observed changes. Thus, I was able to unify the behavioural findings of **Chapter 4** with the neural correlates in **Chapter 5**, placing the LC-NE system or central entrainment as a potential mechanisms, as has been shown previously (Asamoah et al., 2019; Vanneste et al., 2020). Lastly, in the final experiment of **Chapter 6**, I provide evidence of motor cortex excitability changes following NITESGON and a motor learning task, consistent with altered neural plasticity, and adding support to the developing theory of NITESGON's mechanism.

7.2 Further implications

7.2.1 Modulating motor learning in control vs clinical populations

Likely one of the most common limitation of research performed in academic institutes and universities is the homogeneity of student-based samples (Peterson & Merunka, 2014; Sears, 1986). Two of the experiments in this thesis were performed on individuals samples recruited from a higher-level institution, and thus it is necessary to state that replications in external samples is critical to better understand how NITESGON may modulate motor learning. One very prominent difference which must considered, for example, is the effect of age on performance of a serial reaction time task (Hoff et al., 2015; Voelcker-Rehage, 2008). Older adults have been shown have slower response times compared to a younger population (Hoff et al., 2015), and imaging studies have found that older adults exhibit decreased white matter volume in motor regions (Lebel et al., 2012). There is even evidence of altered LC activity as a result of aging, which poses more questions about the effectiveness of NITESGON in an older population (Ludwig et al., 2024). It is therefore important to consider these findings in the context of a younger and academically-based population. Despite these aging aspects, however, there is evidence that NITESGON is effective in older population in the memory domain (Luckey et al., 2020), and so it remains to be seen how motor-paired NITESGON will translate.

Much of the present tACS motor research is conducted in healthy individuals, and while research has used tACS in clinical populations for stroke recovery (Lefebvre et al., 2013; Meng et al., 2024), the proposed mechanism and indeed *goal* of tACS naturally differs between these populations (Yang et al., 2023). In the context of stroke, tACS may act by enhancing the hemodynamics of an affected region, resulting in increased cerebral flow and subsequent recovery of function (Yamamoto et al., 1993). Indeed additional research has shown that tACS can modulate functional communication between brain regions (Yuan et al., 2022). More generally, inducing neuroplasticity has been shown as a promising method for treating stroke (Aderinto et al., 2023), and so mechanistically the presence of NITESGON-induced plasticity would support potential testing for future inclusion in stroke treatment protocols.

NITESGON is particularly unique because of the proposed route by which it elicits its effects. Here, I have provided evidence that NITESGON appears to function transcutaneously (as supported by reduced learning modulation following a nerve block), and that the neural and physiological changes are consistent with altered LC activity. These findings are consistent with other NITESGON work which similarly showed that LC activity and LC-NE activity appear modulated as a result (Vanneste et al., 2020). Following this evidence would mean that the behavioural improvement in sequence acquisition and inter-manual transfer are not from regionally-stimulated effects, but from an overall increase in cortical gain and thus neural plasticity (supported with MEP modulation in **Chapter 6**). In the context of stroke, where motor lesions are variable in size and location, personalization of tACS would be critical when considering the impact on induced electrical fields (Evans et al., 2023). NITESGON instead would be an approach which does not rely on electrode configuration optimization, but instead on non-specific activation of the LC-NE system, and thus may be more practical and universally applicable in individuals with unaffected subcortical regions. Rodent models of peripheral nerve stimulation such as vagus nerve stimulation have shown a specific property which could be described as paired plasticity, whereby stimulation alone is not sufficient to modulate reorganization of brain regions, but also the engagement of these regions. In one study, pairing

auditory tones with VNS drove reorganization of the tonotopic map, however VNS alone did not exhibit this response, leading to the conclusion that transient bursts of neuromodulators via VNS label specific stimuli dependent on engagement and simultaneous exposure (Hays et al., 2013). It is then generally agreed that VNS is largely restricted to the targeted modality and behaviour in question, and thus rehabilitation paradigms would benefit from selecting exercises spanning an optimal and ranging set of behaviours which could be concurrent modulated (Hays et al., 2023).

7.2.3 The implications for transcranial stimulation protocols

Considering that NITESGON resulted in measurable improvement in motor learning behaviour, but without measurable changes in EEG synchronization or power modulation, there is need to discuss how these findings, particularly within the context of previous works (Asamoah et al., 2019; van Boekholdt et al., 2021), might alter usage of current tES protocols. The results of this work do not, however discredit or undermine other findings of transcranial effects such as entrainment (Helfrich et al., 2014; Otero et al., 2020), but rather they provide additional considerations for researchers using this method. Transcranial effects are likely still present in tES research, even in our own findings (albeit lidocaine removed a large component of the effect of the 20 Hz NITESON on learned skill). As a potential addition to future research, tES protocols could consider additional controls such as lidocaine/prilocaine nerve blocks under the region of interest to determine the extent to which transcutaneous effects may driving the results. Such an additional would be beneficial since it would both reduce sensation in the participant (assisting in effective blinding or discomfort), and also inform the researcher on potential mechanisms. When taking the transcutaneous route into consideration, there are also numerous physiological markers which could be used to determine the contributions of arousal to any observed effect such as pupillometry, EEG measures, and biological samples (such markers have already been employed in research, see Gebodh et al. (2024)).

Research into the peripheral effect of tES stimulation is still nascent, and only a handful of works (Asamoah et al., 2019; Gebodh et al., 2024; Luckey, McLeod, Huang, et al., 2022;

Luckey, McLeod, Mohan, & Vanneste, 2022; Vanneste et al., 2020) have directly tested how tACS and tDCS protocols may modulate behaviour and neural activity through peripheral means. As such, there is a substantial body of pre-theory work in tACS in motor modulation alone which could be reconsidered with a peripheral counter-theory, or at minimum, concurrent mechanism in the periphery (Byczynski & Vanneste, 2023).

7.2.4 Integrating NITESON and adaptive motor learning

Discussed extensively in this thesis is the relationship between NITESGON, modulated LC activity, and the adaptive motor learning framework. Established in **Chapter 3**, there is consistent activation of the temporal gyri, motor areas, cerebellum, putamen, pallidum, and thalamus across online motor learning studies. We also established in the literature that these regions are causally involved in adaptive motor learning processes such as error correction and forward model development, movement initiation and control, and later storage and strengthening of the internal model. Accordingly, the role of the LC in modulating adaptive motor learning processes such as neural gain and input weighting (i.e., sensory vs internal) is relevant as we consider how NITESGON might influence these processes through altered LC activity. Consistent with LC-driven cortical gain changes, we observed reduced neural responses after training which were subsequently modulated by 20 Hz stimulation, consistent with the proposed changes that would result from LC output increases during adaptive learning processes. LC involvement was then further supported by altered pupil responses following stimulation at 20 Hz compared to the sham group. We were initially surprised by the observed decreases in pupil size from pre to post 20 Hz stimulation and training which were not seen in the sham group in **Chapter 5**. Further investigation however, revealed that this decrease should reflect compensatory activity following prolonged LC activity increases. In rodent work, behavioural arrests lasting 15 s following 20 s stimulation of the LC (at between 5-20 Hz) was observed and related pharmacologically to lower NE (Harley & Yuan, 2021). Further evidence was also reported in (Carter et al., 2010), where sustained activation of the LC leads to NE exhaustion (Harley & Yuan, 2021), producing altered NE levels following stimulation. It could therefore be

that, following continued NITESGON, NE exhaustion or compensatory LC silencing result in a reduction in central NE and thus reduced sympathetic activity (measured as relative pupil constriction). Following this interpretation, it then appears that NITSGON modulates LC activity and subsequently NE levels in the brain, leading to modulated neural gain (Lee et al., 2018) and thus behavioural improvement through adaptive motor learning processes (Aston-Jones & Cohen, 2005a, 2005b; Mather et al., 2016).

7.3 Future work and next steps

7.3.1 Precision medicine and personalization

One methodological advantage of tACS is that its numerous parameters allow it to be extensively customized and personalized to individuals (Frohlich & Riddle, 2021). Individualized protocols for tACS in Parkinson's patients has already successfully improved motor and cognitive abilities, owed to a greater efficacy of modulation when stimulation is selected in the context of endogenous oscillations (or their subsequent pathology) (Del Felice et al., 2019). Furthermore, current tACS work has been recontextualized with acknowledgement of how individual electric field distribution may impact tACS efficacy (Kasten et al., 2019). Thus, there are numerous sources of individual variability which influence both the mechanism and efficacy of tACS effects. One benefit of NITESGON is that, following the theory that its effects are delivered transcutaneously, there is a reduced influence of aspects such as cranial thickness (influencing field intensity), and here we did not identify any significant effect of resting beta oscillations on the efficacy of NITESGON on degree behavioural modulation. LC-specific stimulation frequencies are generally unstudied in humans given a lack of feasibility to directly stimulate the brainstem, and thus the next steps of this work beyond further establishing the mechanism, will be to understand how different frequency, stimulus intensity, and phase may alter behavioural modulation. Here we have already established frequency-specific effects at 20 Hz, however individual LC differences or more optimal frequencies may exist, positing future research into personalization of NITESGON intervention (Carter et al., 2010; Tseng et al., 2024).

7.4 Conclusion

As further methodological and theoretical constraints arise in tES research for motor learning, alternative hypotheses are necessary to stimulate advancement in the understanding of its mechanism. The peripheral effect hypothesis represents a feasible alternative explanation and method for modulating neural activity through LC and brainstem activity. In this thesis, I sequentially found that stimulating the occipital nerve modulates motor learning behaviour via a transcutaneous mechanism. These changes were correlated with neural correlates in the brain, and physiological measures of arousal. I also present preliminary evidence of modulated neural plasticity, further supporting the involvement of the LC-NE system in NITESGON's mechanism of action. Conclusively, this thesis presents the first evidence of motor learning modulation in humans using the greater occipital nerve. Future work will investigate further the mechanism of NITESGON, the regions implicated in stimulation, the optimization of intervention parameters to individual biology, and its eventual feasibility in clinical populations. Successful implementation of this method would represent a novel treatment for stroke recovery and motor dysfunction, adding to the growing advancement and development of clinical treatment protocols using brain and nerve stimulation.

8 References

- Adair, D., Truong, D., Esmaeilpour, Z., Gebodh, N., Borges, H., Ho, L., Bremner, J. D., Badran, B. W., Napadow, V., & Clark, V. P. (2020). Electrical stimulation of cranial nerves in cognition and disease. *Brain Stimulation*, 13(3), 717-750.
- Adcock, K. S., Lawlor, B., Robertson, I. H., & Vanneste, S. (2022). Diminishing accelerated long-term forgetting in mild cognitive impairment: Study protocol for a prospective, double-blind, placebo-controlled, randomized controlled trial. *Contemporary Clinical Trials Communications*, 30, 100989. <https://doi.org/https://doi.org/10.1016/j.conctc.2022.100989>
- Adelhöfer, N., Mückschel, M., Teufert, B., Ziemssen, T., & Beste, C. (2019). Anodal tDCS affects neuromodulatory effects of the norepinephrine system on superior frontal theta activity during response inhibition. *Brain Structure and Function*, 224(3), 1291-1300.
- Aderinto, N., AbdulBasit, M. O., Olatunji, G., & Adejumo, T. (2023). Exploring the transformative influence of neuroplasticity on stroke rehabilitation: a narrative review of current evidence. *Annals of Medicine and Surgery*, 85(9), 4425-4432.
- Akerman, S., Holland, P. R., & Goadsby, P. J. (2011). Diencephalic and brainstem mechanisms in migraine. *Nature Reviews Neuroscience*, 12(10), 570-584.
- Akkad, H., Dupont-Hadwen, J., Kane, E., Evans, C., Barrett, L., Frese, A., Tetkovic, I., Bestmann, S., & Stagg, C. J. (2021). Increasing human motor skill acquisition by driving theta–gamma coupling. *eLife*, 10, e67355.
- Albouy, G., Sterpenich, V., Balteau, E., Vandewalle, G., Desseilles, M., Dang-Vu, T., Darsaud, A., Ruby, P., Luppi, P. H., Degueldre, C., Peigneux, P., Luxen, A., & Maquet, P. (2008). Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron*, 58(2), 261-272. <https://doi.org/10.1016/j.neuron.2008.02.008>
- Amadi, U., Allman, C., Johansen-Berg, H., & Stagg, C. J. (2015). The Homeostatic Interaction Between Anodal Transcranial Direct Current Stimulation and Motor Learning in Humans is Related to GABAA Activity. *Brain Stimulation*, 8(5), 898-905. <https://doi.org/https://doi.org/10.1016/j.brs.2015.04.010>
- Andersen, K. W., & Siebner, H. R. (2018). Mapping dexterity and handedness: recent insights and future challenges. *Current Opinion in Behavioral Sciences*, 20, 123-129. <https://doi.org/https://doi.org/10.1016/j.cobeha.2017.12.020>

- Angela, J. Y., & Dayan, P. (2005). Uncertainty, neuromodulation, and attention. *Neuron*, 46(4), 681-692.
- Anguera, J. A., Russell, C. A., Noll, D. C., & Seidler, R. D. (2007). Neural correlates associated with intermanual transfer of sensorimotor adaptation. *Brain research*, 1185, 136-151.
- Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., & Paulus, W. (2008). Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. *Brain Stimulation*, 1(2), 97-105.
- Antal, A., & Paulus, W. (2013). Transcranial alternating current stimulation (tACS) [Review]. *Frontiers in Human Neuroscience*, 7. <https://doi.org/10.3389/fnhum.2013.00317>
- Arima, T., Yanagi, Y., Niddam, D. M., Ohata, N., Arendt-Nielsen, L., Minagi, S., Sessle, B. J., & Svensson, P. (2011). Corticomotor plasticity induced by tongue-task training in humans: a longitudinal fMRI study. *Exp Brain Res*, 212(2), 199-212. <https://doi.org/10.1007/s00221-011-2719-7>
- Arnal, L. H., & Giraud, A.-L. (2012). Cortical oscillations and sensory predictions. *Trends in Cognitive Sciences*, 16(7), 390-398. <https://doi.org/https://doi.org/10.1016/j.tics.2012.05.003>
- Asamoah, B., Khatoun, A., & Mc Laughlin, M. (2019). tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. *Nature communications*, 10.
- Aston-Jones, G., & Cohen, J. D. (2005a). Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. *J Comp Neurol*, 493(1), 99-110. <https://doi.org/10.1002/cne.20723>
- Aston-Jones, G., & Cohen, J. D. (2005b). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annu Rev Neurosci*, 28, 403-450. <https://doi.org/10.1146/annurev.neuro.28.061604.135709>
- Attwell, P. J. E., Cooke, S. F., & Yeo, C. H. (2002). Cerebellar Function in Consolidation of a Motor Memory. *Neuron*, 34(6), 1011-1020. [https://doi.org/10.1016/S0896-6273\(02\)00719-5](https://doi.org/10.1016/S0896-6273(02)00719-5)
- Bachtiar, V., Near, J., Johansen-Berg, H., & Stagg, C. J. (2015). Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. *eLife*, 4, e08789. <https://doi.org/10.7554/eLife.08789>

- Barone, J., & Rossiter, H. E. (2021). Understanding the role of sensorimotor beta oscillations. *Frontiers in systems neuroscience*, *15*, 655886.
- Bastani, A., & Jaberzadeh, S. (2012). Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis. *Clinical Neurophysiology*, *123*(4), 644-657.
<https://doi.org/https://doi.org/10.1016/j.clinph.2011.08.029>
- Baumgarten, T. J., Oeltzschner, G., Hoogenboom, N., Wittsack, H. J., Schnitzler, A., & Lange, J. (2016). Beta Peak Frequencies at Rest Correlate with Endogenous GABA+/Cr Concentrations in Sensorimotor Cortex Areas. *PLOS ONE*, *11*(6), e0156829. <https://doi.org/10.1371/journal.pone.0156829>
- Bestmann, S., & Krakauer, J. W. (2015). The uses and interpretations of the motor-evoked potential for understanding behaviour. *Experimental brain research*, *233*(3), 679-689. <https://doi.org/10.1007/s00221-014-4183-7>
- Birbaumer, N. (2007). Motor Learning: Passing a Skill from One Hand to the Other. *Current Biology*, *17*(23), R1024-R1026.
<https://doi.org/https://doi.org/10.1016/j.cub.2007.10.004>
- Bland, N. S. (2021). Is two decades of tDCS work wrong? Commentary on Ahn and Frohlich. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, *14*(2), 438-439. <https://doi.org/10.1016/j.brs.2021.02.016>
- Bo, J., Peltier, S. J., Noll, D. C., & Seidler, R. D. (2011). Symbolic representations in motor sequence learning. *Neuroimage*, *54*(1), 417-426.
<https://doi.org/https://doi.org/10.1016/j.neuroimage.2010.08.019>
- Bologna, M., Guerra, A., Paparella, G., Colella, D., Borrelli, A., Suppa, A., Di Lazzaro, V., Brown, P., & Berardelli, A. (2019). Transcranial Alternating Current Stimulation Has Frequency-Dependent Effects on Motor Learning in Healthy Humans. *Neuroscience*, *411*, 130-139.
<https://doi.org/https://doi.org/10.1016/j.neuroscience.2019.05.041>
- Bönstrup, M., Iturrate, I., Thompson, R., Cruciani, G., Censor, N., & Cohen, L. G. (2019). A Rapid Form of Offline Consolidation in Skill Learning. *Current Biology*, *29*(8), 1346-1351.e1344. <https://doi.org/10.1016/j.cub.2019.02.049>
- Boyce, R., Glasgow, S. D., Williams, S., & Adamantidis, A. (2016). Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science*, *352*(6287), 812-816. <https://doi.org/doi:10.1126/science.aad5252>

- Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Progress in Neurobiology*, 76(2), 99-125. <https://doi.org/10.1016/j.pneurobio.2005.06.003>
- Broncel, A., Bocian, R., Kłos-Wojtczak, P., & Konopacki, J. (2019). GABAergic mediation of hippocampal theta rhythm induced by stimulation of the vagal nerve. *Brain Research Bulletin*, 147, 110-123. <https://doi.org/10.1016/j.brainresbull.2019.02.010>
- Brougher, J., Aziz, U., Adari, N., Chaturvedi, M., Jules, A., Shah, I., Syed, S., & Thorn, C. A. (2021). Self-Administration of Right Vagus Nerve Stimulation Activates Midbrain Dopaminergic Nuclei. *Front Neurosci*, 15, 782786. <https://doi.org/10.3389/fnins.2021.782786>
- Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., Edwards, D. J., Valero-Cabre, A., Rotenberg, A., Pascual-Leone, A., Ferrucci, R., Priori, A., Boggio, P. S., & Fregni, F. (2012). Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions. *Brain Stimulation*, 5(3), 175-195. <https://doi.org/10.1016/j.brs.2011.03.002>
- Buch, E. R., Santarnecchi, E., Antal, A., Born, J., Celnik, P. A., Classen, J., Gerloff, C., Hallett, M., Hummel, F. C., Nitsche, M. A., Pascual-Leone, A., Paulus, W. J., Reis, J., Robertson, E. M., Rothwell, J. C., Sandrini, M., Schambra, H. M., Wassermann, E. M., Ziemann, U., & Cohen, L. G. (2017). Effects of tDCS on motor learning and memory formation: A consensus and critical position paper. *Clin Neurophysiol*, 128(4), 589-603. <https://doi.org/10.1016/j.clinph.2017.01.004>
- Buchanan, D. M., D'Angiulli, A., Samson, A., Maisonneuve, A. R., & Robaey, P. (2022). Acceptability of transcranial direct current stimulation in children and adolescents with ADHD: The point of view of parents. *Journal of Health Psychology*, 27(1), 36-46. <https://doi.org/10.1177/1359105320937059>
- Buchanan, D. M., Robaey, P., & D'Angiulli, A. (2020). What Do We Know about Transcranial Direct Current Stimulation for Major Depression? *Brain Sciences*, 10(8), 480. <https://www.mdpi.com/2076-3425/10/8/480>
- Buell, E. P., Loerwald, K. W., Engineer, C. T., Borland, M. S., Buell, J. M., Kelly, C. A., Khan, I. I., Hays, S. A., & Kilgard, M. P. (2018). Cortical map plasticity as a

- function of vagus nerve stimulation rate. *Brain Stimulation*, 11(6), 1218-1224.
<https://doi.org/https://doi.org/10.1016/j.brs.2018.07.045>
- Burkhardt, G., Goerigk, S., & Padberg, F. (2024). How effective is transcranial direct current stimulation? – Authors' reply. *The Lancet*, 403(10445), 2689-2690. [https://doi.org/10.1016/S0140-6736\(24\)00635-4](https://doi.org/10.1016/S0140-6736(24)00635-4)
- Byczynski, G., & Vanneste, S. (2023). Modulating motor learning with brain stimulation: Stage-specific perspectives for transcranial and transcutaneous delivery. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 125, 110766. <https://doi.org/https://doi.org/10.1016/j.pnpbp.2023.110766>
- Cao, L., & Hu, Y.-M. (2016). Beta Rebound in Visuomotor Adaptation: Still the Status Quo? *The Journal of Neuroscience*, 36(24), 6365-6367.
<https://doi.org/10.1523/jneurosci.1007-16.2016>
- Carter, M. E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., & de Lecea, L. (2010). Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nature Neuroscience*, 13(12), 1526-1533. <https://doi.org/10.1038/nn.2682>
- Caulfield, K. A., Fleischmann, H. H., Cox, C. E., Wolf, J. P., George, M. S., & McTeague, L. M. (2022). Neuronavigation maximizes accuracy and precision in TMS positioning: Evidence from 11,230 distance, angle, and electric field modeling measurements. *Brain Stimul*, 15(5), 1192-1205.
<https://doi.org/10.1016/j.brs.2022.08.013>
- Cavaleri, R., Schabrun, S. M., & Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Systematic Reviews*, 6(1), 48.
<https://doi.org/10.1186/s13643-017-0440-8>
- Cha, T. H., & Hwang, H. S. (2022). Rehabilitation Interventions Combined with Noninvasive Brain Stimulation on Upper Limb Motor Function in Stroke Patients. *Brain Sci*, 12(8). <https://doi.org/10.3390/brainsci12080994>
- Chan, M. M. Y., Yau, S. S. Y., & Han, Y. M. Y. (2021). The neurobiology of prefrontal transcranial direct current stimulation (tDCS) in promoting brain plasticity: A systematic review and meta-analyses of human and rodent studies. *Neuroscience & Biobehavioral Reviews*, 125, 392-416.
<https://doi.org/https://doi.org/10.1016/j.neubiorev.2021.02.035>

- Chase, C., & Seidler, R. (2008). Degree of handedness affects intermanual transfer of skill learning. *Exp Brain Res*, 190(3), 317-328. <https://doi.org/10.1007/s00221-008-1472-z>
- Christova, M., Rafolt, D., & Gallasch, E. (2015). Cumulative effects of anodal and priming cathodal tDCS on pegboard test performance and motor cortical excitability. *Behavioural Brain Research*, 287, 27-33. <https://doi.org/https://doi.org/10.1016/j.bbr.2015.03.028>
- Ciechanski, P., & Kirton, A. (2017). Transcranial Direct-Current Stimulation Can Enhance Motor Learning in Children. *Cerebral Cortex*, 27(5), 2758-2767. <https://doi.org/10.1093/cercor/bhw114>
- Cirillo, J., Todd, G., & Semmler, J. G. (2011). Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. *European Journal of Neuroscience*, 34(11), 1847-1856. <https://doi.org/https://doi.org/10.1111/j.1460-9568.2011.07870.x>
- Cobia, D., Haut, M. W., Revill, K. P., Rellick, S. L., Nudo, R. J., Wischniewski, M., & Buetefisch, C. M. (2024). Gray matter volume of functionally relevant primary motor cortex is causally related to learning a hand motor task. *Cereb Cortex*, 34(5). <https://doi.org/10.1093/cercor/bhae210>
- Cohen, D. A., Pascual-Leone, A., Press, D. Z., & Robertson, E. M. (2005). Off-line learning of motor skill memory: A double dissociation of goal and movement. *Proceedings of the National Academy of Sciences*, 102(50), 18237-18241. <https://doi.org/doi:10.1073/pnas.0506072102>
- Cohen, L. G., Ziemann, U., Chen, R., Classen, J., Hallett, M., Gerloff, C., & Butefisch, C. (1998). Studies of Neuroplasticity With Transcranial Magnetic Stimulation. *Journal of Clinical Neurophysiology*, 15(4), 305-324. https://journals.lww.com/clinicalneurophys/fulltext/1998/07000/studies_of_neuroplasticity_with_transcranial.3.aspx
- Conessa, A., Debarnot, U., Siegler, I., & Boutin, A. (2023). Sleep-related motor skill consolidation and generalizability after physical practice, motor imagery, and action observation. *iScience*, 26(8). <https://doi.org/10.1016/j.isci.2023.107314>
- Cunningham, D. A., Machado, A., Yue, G. H., Carey, J. R., & Plow, E. B. (2013). Functional somatotopy revealed across multiple cortical regions using a model of complex motor task. *Brain Res*, 1531, 25-36. <https://doi.org/10.1016/j.brainres.2013.07.050>

- Dahms, C., Brodoehl, S., Witte, O. W., & Klingner, C. M. (2020). The importance of different learning stages for motor sequence learning after stroke. *Human Brain Mapping, 41*(1), 270-286. <https://doi.org/https://doi.org/10.1002/hbm.24793>
- Dawson, J., Pierce, D., Dixit, A., Kimberley, T. J., Robertson, M., Tarver, B., Hilmi, O., McLean, J., Forbes, K., Kilgard, M. P., Rennaker, R. L., Cramer, S. C., Walters, M., & Engineer, N. (2016). Safety, Feasibility, and Efficacy of Vagus Nerve Stimulation Paired With Upper-Limb Rehabilitation After Ischemic Stroke. *Stroke, 47*(1), 143-150. <https://doi.org/doi:10.1161/STROKEAHA.115.010477>
- Dayan, E., & Cohen, L. G. (2011). Neuroplasticity subserving motor skill learning. *Neuron, 72*(3), 443-454.
- De Ridder, D., Vanneste, S., Engineer, N. D., & Kilgard, M. P. (2014). Safety and Efficacy of Vagus Nerve Stimulation Paired With Tones for the Treatment of Tinnitus: A Case Series. *Neuromodulation: Technology at the Neural Interface, 17*(2), 170-179. <https://doi.org/https://doi.org/10.1111/ner.12127>
- Debas, K., Carrier, J., Barakat, M., Marrelec, G., Bellec, P., Tahar, A. H., Karni, A., Ungerleider, L. G., Benali, H., & Doyon, J. (2014). Off-line consolidation of motor sequence learning results in greater integration within a cortico-striatal functional network. *NeuroImage, 99*, 50-58.
- Debas, K., Carrier, J., Orban, P., Barakat, M., Lungu, O., Vandewalle, G., Tahar, A. H., Bellec, P., Karni, A., & Ungerleider, L. G. (2010). Brain plasticity related to the consolidation of motor sequence learning and motor adaptation. *Proceedings of the National Academy of Sciences, 107*(41), 17839-17844.
- Del Felice, A., Castiglia, L., Formaggio, E., Cattelan, M., Scarpa, B., Manganotti, P., Tenconi, E., & Masiero, S. (2019). Personalized transcranial alternating current stimulation (tACS) and physical therapy to treat motor and cognitive symptoms in Parkinson's disease: A randomized cross-over trial. *Neuroimage Clin, 22*, 101768. <https://doi.org/10.1016/j.nicl.2019.101768>
- Devilbiss, D. M., & Waterhouse, B. D. (2011). Phasic and tonic patterns of locus coeruleus output differentially modulate sensory network function in the awake rat. *J Neurophysiol, 105*(1), 69-87. <https://doi.org/10.1152/jn.00445.2010>
- Di Lazzaro, V., Profice, P., Pilato, F., Capone, F., Ranieri, F., Pasqualetti, P., Colosimo, C., Pravatà, E., Cianfoni, A., & Dileone, M. (2009). Motor Cortex Plasticity Predicts Recovery in Acute Stroke. *Cerebral Cortex, 20*(7), 1523-1528. <https://doi.org/10.1093/cercor/bhp216>

- Di Rienzo, F., Debarnot, U., Daligault, S., Saruco, E., Delpuech, C., Doyon, J., Collet, C., & Guillot, A. (2016). Online and Offline Performance Gains Following Motor Imagery Practice: A Comprehensive Review of Behavioral and Neuroimaging Studies [Review]. *Frontiers in Human Neuroscience*, 10. <https://doi.org/10.3389/fnhum.2016.00315>
- Diedrichsen, J., & Kornysheva, K. (2015). Motor skill learning between selection and execution. *Trends in Cognitive Sciences*, 19(4), 227-233. <https://doi.org/10.1016/j.tics.2015.02.003>
- DiGuseppi, J., & Tadi, P. (2023). Neuroanatomy, Postcentral Gyrus. In *StatPearls*. StatPearls Publishing
- Copyright © 2023, StatPearls Publishing LLC.
- Dirren, E., Bourgeois, A., Klug, J., Kleinschmidt, A., van Assche, M., & Carrera, E. (2021). The neural correlates of intermanual transfer. *NeuroImage*, 245, 118657. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2021.118657>
- Dobkin, B. H. (2004). Strategies for stroke rehabilitation. *The Lancet Neurology*, 3(9), 528-536. [https://doi.org/10.1016/S1474-4422\(04\)00851-8](https://doi.org/10.1016/S1474-4422(04)00851-8)
- Dockès, J., Poldrack, R. A., Primet, R., Gözükan, H., Yarkoni, T., Suchanek, F., Thirion, B., & Varoquaux, G. (2020). NeuroQuery, comprehensive meta-analysis of human brain mapping. *eLife*, 9, e53385. <https://doi.org/10.7554/eLife.53385>
- Doyon, J., & Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Current opinion in neurobiology*, 15(2), 161-167.
- Drobyshevsky, A., Baumann, S. B., & Schneider, W. (2006). A rapid fMRI task battery for mapping of visual, motor, cognitive, and emotional function. *NeuroImage*, 31(2), 732-744. <https://doi.org/10.1016/j.neuroimage.2005.12.016>
- Ebrahimi, S., & Ostry, D. J. (2024). The human somatosensory cortex contributes to the encoding of newly learned movements. *Proceedings of the National Academy of Sciences*, 121(6), e2316294121. <https://doi.org/doi:10.1073/pnas.2316294121>
- Ehsani, F., Bakhtiary, A. H., Jaberzadeh, S., Talimkhani, A., & Hajihassani, A. (2016). Differential effects of primary motor cortex and cerebellar transcranial direct current stimulation on motor learning in healthy individuals: A randomized double-blind sham-controlled study. *Neuroscience Research*, 112, 10-19. <https://doi.org/https://doi.org/10.1016/j.neures.2016.06.003>

- Eickhoff, S. B., Laird, A. R., Grefkes, C., Wang, L. E., Zilles, K., & Fox, P. T. (2009). Coordinate-based activation likelihood estimation meta-analysis of neuroimaging data: A random-effects approach based on empirical estimates of spatial uncertainty. *Human Brain Mapping*, 30(9), 2907-2926.
<https://doi.org/https://doi.org/10.1002/hbm.20718>
- Eisenstein, T., Furman-Haran, E., & Tal, A. (2024). Early excitatory-inhibitory cortical modifications following skill learning are associated with motor memory consolidation and plasticity overnight. *Nature communications*, 15(1), 906.
<https://doi.org/10.1038/s41467-024-44979-9>
- Espenhahn, S., Rossiter, H. E., van Wijk, B. C., Redman, N., Rondina, J. M., Diedrichsen, J., & Ward, N. S. (2020). Sensorimotor cortex beta oscillations reflect motor skill learning ability after stroke. *Brain communications*, 2(2), fcaa161.
- Evans, C., Johnstone, A., Zich, C., Lee, J. S. A., Ward, N. S., & Bestmann, S. (2023). The impact of brain lesions on tDCS-induced electric fields. *Scientific Reports*, 13(1), 19430. <https://doi.org/10.1038/s41598-023-45905-7>
- Fang, Z., Sack, A. T., & Leunissen, I. (2024). The phase of tACS-entrained pre-SMA beta oscillations modulates motor inhibition. *NeuroImage*, 290, 120572.
<https://doi.org/https://doi.org/10.1016/j.neuroimage.2024.120572>
- Fernández-Seara, M. A., Aznárez-Sanado, M., Mengual, E., Loayza, F. R., & Pastor, M. A. (2009). Continuous performance of a novel motor sequence leads to highly correlated striatal and hippocampal perfusion increases. *NeuroImage*, 47(4), 1797-1808.
- Feulner, B., Perich, M. G., Chowdhury, R. H., Miller, L. E., Gallego, J. A., & Clopath, C. (2022). Small, correlated changes in synaptic connectivity may facilitate rapid motor learning. *Nature communications*, 13(1), 5163.
<https://doi.org/10.1038/s41467-022-32646-w>
- Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., & Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *Journal of Neuroscience*, 33(44), 17483-17489.
- Fresnoza, S., Christova, M., Bieler, L., Körner, C., Zimmer, U., Gallasch, E., & Ischebeck, A. (2020). Age-Dependent Effect of Transcranial Alternating Current Stimulation on Motor Skill Consolidation. *Front Aging Neurosci*, 12, 25.
<https://doi.org/10.3389/fnagi.2020.00025>

- Fresnoza, S., Christova, M., Feil, T., Gallasch, E., Körner, C., Zimmer, U., & Ischebeck, A. (2018). The effects of transcranial alternating current stimulation (tACS) at individual alpha peak frequency (iAPF) on motor cortex excitability in young and elderly adults. *Exp Brain Res*, 236(10), 2573-2588.
<https://doi.org/10.1007/s00221-018-5314-3>
- Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., & Lu, B. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron*, 66(2), 198-204.
- Frohlich, F., & Riddle, J. (2021). Conducting double-blind placebo-controlled clinical trials of transcranial alternating current stimulation (tACS). *Translational Psychiatry*, 11(1), 284. <https://doi.org/10.1038/s41398-021-01391-x>
- Fujiyama, H., Hinder, M. R., Barzideh, A., Van de Vijver, C., Badache, A. C., Manrique-C, M. N., Reissig, P., Zhang, X., Levin, O., Summers, J. J., & Swinnen, S. P. (2017). Preconditioning tDCS facilitates subsequent tDCS effect on skill acquisition in older adults. *Neurobiology of Aging*, 51, 31-42.
<https://doi.org/https://doi.org/10.1016/j.neurobiolaging.2016.11.012>
- Galea, J. M., Vazquez, A., Pasricha, N., Orban de Xivry, J.-J., & Celnik, P. (2010). Dissociating the Roles of the Cerebellum and Motor Cortex during Adaptive Learning: The Motor Cortex Retains What the Cerebellum Learns. *Cerebral Cortex*, 21(8), 1761-1770. <https://doi.org/10.1093/cercor/bhq246>
- García-Magro, N., Negredo, P., Martín, Y. B., Nuñez, Á., & Avendaño, C. (2020). Modulation of mechanosensory vibrissal responses in the trigeminocervical complex by stimulation of the greater occipital nerve in a rat model of trigeminal neuropathic pain. *The Journal of Headache and Pain*, 21(1), 96.
<https://doi.org/10.1186/s10194-020-01161-y>
- Gastrock, R. Q., 't Hart, B. M., & Henriques, D. Y. P. (2024). Distinct learning, retention, and generalization patterns in de novo learning versus motor adaptation. *Scientific Reports*, 14(1), 8906. <https://doi.org/10.1038/s41598-024-59445-1>
- Gebodh, N., Miskovic, V., Laszlo, S., Datta, A., & Bikson, M. (2024). Frontal HD-tACS enhances behavioral and EEG biomarkers of vigilance in continuous attention task. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 17(3), 683-686. <https://doi.org/10.1016/j.brs.2024.05.009>

- Ghai, S., Schmitz, G., Hwang, T. H., & Effenberg, A. O. (2019). Training proprioception with sound: effects of real-time auditory feedback on intermodal learning. *Ann N Y Acad Sci*, 1438(1), 50-61. <https://doi.org/10.1111/nyas.13967>
- Gheysen, F., Van Opstal, F., Roggeman, C., Van Waelvelde, H., & Fias, W. (2010). Hippocampal contribution to early and later stages of implicit motor sequence learning. *Experimental brain research*, 202, 795-807.
- Ghilardi, M.-F., Ghez, C., Dhawan, V., Moeller, J., Mentis, M., Nakamura, T., Antonini, A., & Eidelberg, D. (2000). Patterns of regional brain activation associated with different forms of motor learning. *Brain research*, 871(1), 127-145. [https://doi.org/https://doi.org/10.1016/S0006-8993\(00\)02365-9](https://doi.org/https://doi.org/10.1016/S0006-8993(00)02365-9)
- Giustiniani, A., Tarantino, V., Bonaventura, R. E., Smirni, D., Turriziani, P., & Oliveri, M. (2019a). Effects of low-gamma tACS on primary motor cortex in implicit motor learning. *Behav Brain Res*, 376, 112170. <https://doi.org/10.1016/j.bbr.2019.112170>
- Giustiniani, A., Tarantino, V., Bonaventura, R. E., Smirni, D., Turriziani, P., & Oliveri, M. (2019b). Effects of low-gamma tACS on primary motor cortex in implicit motor learning. *Behavioural Brain Research*, 376, 112170. <https://doi.org/https://doi.org/10.1016/j.bbr.2019.112170>
- Giustiniani, A., Tarantino, V., Bracco, M., Bonaventura, R. E., & Oliveri, M. (2021). Functional Role of Cerebellar Gamma Frequency in Motor Sequences Learning: a tACS Study. *The Cerebellum*, 20(6), 913-921. <https://doi.org/10.1007/s12311-021-01255-6>
- Glennon, E., Carcea, I., Martins, A. R. O., Multani, J., Shehu, I., Svirsky, M. A., & Froemke, R. C. (2019). Locus coeruleus activation accelerates perceptual learning. *Brain research*, 1709, 39-49. <https://doi.org/https://doi.org/10.1016/j.brainres.2018.05.048>
- Goodwill, A. M., Teo, W.-P., Morgan, P., Daly, R. M., & Kidgell, D. J. (2016). Bihemispheric-tDCS and Upper Limb Rehabilitation Improves Retention of Motor Function in Chronic Stroke: A Pilot Study [Original Research]. *Frontiers in Human Neuroscience*, 10. <https://doi.org/10.3389/fnhum.2016.00258>
- Grafton, S. T., Schmitt, P., Van Horn, J., & Diedrichsen, J. (2008). Neural substrates of visuomotor learning based on improved feedback control and prediction. *NeuroImage*, 39(3), 1383-1395.

- Gregory, M. D., Agam, Y., Selvadurai, C., Nagy, A., Vangel, M., Tucker, M., Robertson, E. M., Stickgold, R., & Manoach, D. S. (2014). Resting state connectivity immediately following learning correlates with subsequent sleep-dependent enhancement of motor task performance. *NeuroImage*, 102, 666-673.
- Groenewegen, H. J. (2003). The Basal Ganglia and Motor Control. *Neural Plasticity*, 10, 108384. <https://doi.org/10.1155/NP.2003.107>
- Grosbras, M. H., Beaton, S., & Eickhoff, S. B. (2012). Brain regions involved in human movement perception: A quantitative voxel-based meta-analysis. *Human Brain Mapping*, 33(2), 431-454.
- Gryga, M., Taubert, M., Dukart, J., Vollmann, H., Conde, V., Sehm, B., Villringer, A., & Ragert, P. (2012). Bidirectional gray matter changes after complex motor skill learning. *Frontiers in systems neuroscience*, 6, 37.
- Guerra, A., Suppa, A., Asci, F., De Marco, G., D'Onofrio, V., Bologna, M., Di Lazzaro, V., & Berardelli, A. (2019). LTD-like plasticity of the human primary motor cortex can be reversed by γ -tACS. *Brain Stimul*, 12(6), 1490-1499. <https://doi.org/10.1016/j.brs.2019.06.029>
- Gupta, M. W., & Rickard, T. C. (2024). Comparison of online, offline, and hybrid hypotheses of motor sequence learning using a quantitative model that incorporate reactive inhibition. *Scientific Reports*, 14(1), 4661. <https://doi.org/10.1038/s41598-024-52726-9>
- Hamada, M., Hanajima, R., Terao, Y., Arai, N., Furubayashi, T., Inomata-Terada, S., Yugeta, A., Matsumoto, H., Shirota, Y., & Ugawa, Y. (2007). Origin of facilitation in repetitive, 1.5ms interval, paired pulse transcranial magnetic stimulation (rPPS) of the human motor cortex. *Clinical Neurophysiology*, 118(7), 1596-1601. <https://doi.org/https://doi.org/10.1016/j.clinph.2007.03.009>
- Hamano, Y. H., Sugawara, S. K., Fukunaga, M., & Sadato, N. (2021). The integrative role of the M1 in motor sequence learning. *Neuroscience Letters*, 760, 136081. <https://doi.org/https://doi.org/10.1016/j.neulet.2021.136081>
- Hardwick, R. M., Rottschy, C., Miall, R. C., & Eickhoff, S. B. (2013). A quantitative meta-analysis and review of motor learning in the human brain. *NeuroImage*, 67, 283-297. <https://doi.org/10.1016/j.neuroimage.2012.11.020>
- Harley, C. W., & Yuan, Q. (2021). Locus Coeruleus Optogenetic Modulation: Lessons Learned from Temporal Patterns. *Brain Sci*, 11(12). <https://doi.org/10.3390/brainsci11121624>

- Hays, S. A., Rennaker, R. L., & Kilgard, M. P. (2013). Targeting plasticity with vagus nerve stimulation to treat neurological disease. *Prog Brain Res*, 207, 275-299. <https://doi.org/10.1016/b978-0-444-63327-9.00010-2>
- Hays, S. A., Rennaker, R. L., & Kilgard, M. P. (2023). How to fail with paired VNS therapy. *Brain Stimulation*, 16(5), 1252-1258. <https://doi.org/https://doi.org/10.1016/j.brs.2023.08.009>
- Hays, S. A., Ruiz, A., Bethea, T., Khodaparast, N., Carmel, J. B., Rennaker, R. L., & Kilgard, M. P. (2016). Vagus nerve stimulation during rehabilitative training enhances recovery of forelimb function after ischemic stroke in aged rats. *Neurobiology of Aging*, 43, 111-118. <https://doi.org/https://doi.org/10.1016/j.neurobiolaging.2016.03.030>
- Hays, S. A., Ruiz, A., Hulsey, D. R., Khodaparast, N., Bethea, T., Carmel, J. B., Rennaker, R. L., & Kilgard, M. P. (2015). Abstract 162: Vagus Nerve Stimulation Enhances Neuroplasticity and Forelimb Recovery after Stroke in Aged Rats. *Stroke*, 46(suppl_1), A162-A162. https://doi.org/doi:10.1161/str.46.suppl_1.162
- Heinrichs-Graham, E., Kurz, M. J., Gehringer, J. E., & Wilson, T. W. (2017). The functional role of post-movement beta oscillations in motor termination. *Brain Struct Funct*, 222(7), 3075-3086. <https://doi.org/10.1007/s00429-017-1387-1>
- Heitger, M. H., Ronsse, R., Dhollander, T., Dupont, P., Caeyenberghs, K., & Swinnen, S. P. (2012). Motor learning-induced changes in functional brain connectivity as revealed by means of graph-theoretical network analysis. *NeuroImage*, 61(3), 633-650. <https://doi.org/10.1016/j.neuroimage.2012.03.067>
- Helfrich, Randolph F., Schneider, Till R., Rach, S., Trautmann-Lengsfeld, Sina A., Engel, Andreas K., & Herrmann, Christoph S. (2014). Entrainment of Brain Oscillations by Transcranial Alternating Current Stimulation. *Current Biology*, 24(3), 333-339. <https://doi.org/https://doi.org/10.1016/j.cub.2013.12.041>
- Hermanns, H., Hollmann, M. W., Stevens, M. F., Lirk, P., Brandenburger, T., Piegeler, T., & Werdehausen, R. (2019). Molecular mechanisms of action of systemic lidocaine in acute and chronic pain: a narrative review. *British Journal of Anaesthesia*, 123(3), 335-349. <https://doi.org/https://doi.org/10.1016/j.bja.2019.06.014>
- Herrmann, C., Rach, S., Neuling, T., & Strüber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of

- cognitive processes [Review]. *Frontiers in Human Neuroscience*, 7.
<https://doi.org/10.3389/fnhum.2013.00279>
- Hétu, S., Grégoire, M., Saimpont, A., Coll, M.-P., Eugène, F., Michon, P.-E., & Jackson, P. L. (2013). The neural network of motor imagery: An ALE meta-analysis. *Neuroscience & Biobehavioral Reviews*, 37(5), 930-949.
<https://doi.org/https://doi.org/10.1016/j.neubiorev.2013.03.017>
- Heun, R., Freymann, N., Granath, D. O., Stracke, C. P., Jessen, F., Barkow, K., & Reul, J. (2004). Differences of cerebral activation between superior and inferior learners during motor sequence encoding and retrieval. *Psychiatry Res*, 132(1), 19-32. <https://doi.org/10.1016/j.psychresns.2004.01.007>
- Hoff, M., Trapp, S., Kaminski, E., Sehm, B., Steele, C. J., Villringer, A., & Ragert, P. (2015). Switching between hands in a serial reaction time task: a comparison between young and old adults [Original Research]. *Frontiers in Aging Neuroscience*, 7. <https://doi.org/10.3389/fnagi.2015.00176>
- Horiba, M., Ueki, Y., Nojima, I., Shimizu, Y., Sahashi, K., Itamoto, S., Suzuki, A., Yamada, G., Matsukawa, N., & Wada, I. (2019). Impaired motor skill acquisition using mirror visual feedback improved by transcranial direct current stimulation (tDCS) in patients with Parkinson's disease. *Frontiers in neuroscience*, 13, 602.
- Huang, Vincent S., Haith, A., Mazzoni, P., & Krakauer, John W. (2011). Rethinking Motor Learning and Savings in Adaptation Paradigms: Model-Free Memory for Successful Actions Combines with Internal Models. *Neuron*, 70(4), 787-801.
<https://doi.org/https://doi.org/10.1016/j.neuron.2011.04.012>
- Huang, Y., Liu, A. A., Lafon, B., Friedman, D., Dayan, M., Wang, X., Bikson, M., Doyle, W. K., Devinsky, O., & Parra, L. C. (2017). Measurements and models of electric fields in the in vivo human brain during transcranial electric stimulation. *eLife*, 6, e18834. <https://doi.org/10.7554/eLife.18834>
- Hulsey, D. R., Hays, S. A., Khodaparast, N., Ruiz, A., Das, P., Rennaker II, R. L., & Kilgard, M. P. (2016). Reorganization of motor cortex by vagus nerve stimulation requires cholinergic innervation. *Brain Stimulation*, 9(2), 174-181.
- Hulsey, D. R., Hays, S. A., Khodaparast, N., Ruiz, A., Das, P., Rennaker, R. L., & Kilgard, M. P. (2016). Reorganization of Motor Cortex by Vagus Nerve Stimulation Requires Cholinergic Innervation. *Brain Stimulation*, 9(2), 174-181.
<https://doi.org/https://doi.org/10.1016/j.brs.2015.12.007>

- Hulsey, D. R., Riley, J. R., Loerwald, K. W., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2017). Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. *Experimental Neurology*, 289, 21-30. <https://doi.org/10.1016/j.expneurol.2016.12.005>
- Hulsey, D. R., Shedd, C. M., Sarker, S. F., Kilgard, M. P., & Hays, S. A. (2019). Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. *Experimental Neurology*, 320, 112975. <https://doi.org/10.1016/j.expneurol.2019.112975>
- Ibáñez, J., Zicher, B., Brown, K. E., Rocchi, L., Casolo, A., Del Vecchio, A., Spampinato, D., Vollette, C.-A., Rothwell, J. C., Baker, S. N., & Farina, D. (2023). Standard intensities of transcranial alternating current stimulation over the motor cortex do not entrain corticospinal inputs to motor neurons. *The Journal of physiology*, 601(15), 3187-3199. <https://doi.org/10.1113/JP282983>
- Ito, T., Coppola, J. H., & Ostry, D. J. (2016). Speech motor learning changes the neural response to both auditory and somatosensory signals. *Scientific Reports*, 6(1), 25926. <https://doi.org/10.1038/srep25926>
- Iwama, S., Yanagisawa, T., Hirose, R., & Ushiba, J. (2022). Beta rhythmicity in human motor cortex reflects neural population coupling that modulates subsequent finger coordination stability. *Communications Biology*, 5(1), 1375. <https://doi.org/10.1038/s42003-022-04326-4>
- Jacobson, L., Koslowsky, M., & Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Experimental brain research*, 216(1), 1-10.
- JASP. (2024). *JASP (Version 0.18.3)*. In <https://jasp-stats.org/>
- Jie, L. J., Kleynen, M., Meijer, K., Beurskens, A., & Braun, S. (2018). The Effects of Implicit and Explicit Motor Learning in Gait Rehabilitation of People After Stroke: Protocol for a Randomized Controlled Trial. *JMIR Res Protoc*, 7(5), e142. <https://doi.org/10.2196/resprot.9595>
- Jigo, M., Carmel, J. B., Wang, Q., & Rodenkirch, C. (2024). Transcutaneous cervical vagus nerve stimulation improves sensory performance in humans: a randomized controlled crossover pilot study. *Scientific Reports*, 14(1), 3975. <https://doi.org/10.1038/s41598-024-54026-8>

- Jo, J. M., Kim, Y.-H., Ko, M.-H., Ohn, S. H., Joen, B., & Lee, K. H. (2009). Enhancing the working memory of stroke patients using tDCS. *American Journal of Physical Medicine & Rehabilitation*, 88(5), 404-409.
- Jochumsen, M., Roving, C., Roving, H., Cremoux, S., Signal, N., Allen, K., Taylor, D., & Niazi, I. K. (2017). Quantification of Movement-Related EEG Correlates Associated with Motor Training: A Study on Movement-Related Cortical Potentials and Sensorimotor Rhythms. *Front Hum Neurosci*, 11, 604.
<https://doi.org/10.3389/fnhum.2017.00604>
- Jordan, R. (2024). The locus coeruleus as a global model failure system. *Trends in Neurosciences*, 47(2), 92-105. <https://doi.org/10.1016/j.tins.2023.11.006>
- Jurkiewicz, M. T., Gaetz, W. C., Bostan, A. C., & Cheyne, D. (2006). Post-movement beta rebound is generated in motor cortex: evidence from neuromagnetic recordings. *NeuroImage*, 32(3), 1281-1289.
<https://doi.org/10.1016/j.neuroimage.2006.06.005>
- Kal, E., Prosée, R., Winters, M., & van der Kamp, J. (2018). Does implicit motor learning lead to greater automatization of motor skills compared to explicit motor learning? A systematic review. *PLOS ONE*, 13(9), e0203591.
<https://doi.org/10.1371/journal.pone.0203591>
- Karim, H. T., Huppert, T. J., Erickson, K. I., Wollam, M. E., Sparto, P. J., Sejdić, E., & VanSwearingen, J. M. (2017). Motor sequence learning-induced neural efficiency in functional brain connectivity. *Behavioural Brain Research*, 319, 87-95.
- Kasten, F. H., Dowsett, J., & Herrmann, C. S. (2016). Sustained Aftereffect of α -tACS Lasts Up to 70 min after Stimulation [Original Research]. *Frontiers in Human Neuroscience*, 10. <https://doi.org/10.3389/fnhum.2016.00245>
- Kasten, F. H., Duecker, K., Maack, M. C., Meiser, A., & Herrmann, C. S. (2019). Integrating electric field modeling and neuroimaging to explain inter-individual variability of tACS effects. *Nature communications*, 10(1), 5427.
<https://doi.org/10.1038/s41467-019-13417-6>
- Kennedy, D. M., Wang, C., Wang, Y., & Shea, C. H. (2021). The influence of accuracy constraints on bimanual and unimanual sequence learning. *Neuroscience Letters*, 751, 135812.
<https://doi.org/https://doi.org/10.1016/j.neulet.2021.135812>

- Khedr, E. M., Shawky, O. A., El-Hammady, D. H., Rothwell, J. C., Darwish, E. S., Mostafa, O. M., & Tohamy, A. M. (2013). Effect of anodal versus cathodal transcranial direct current stimulation on stroke rehabilitation: a pilot randomized controlled trial. *Neurorehabilitation and neural repair*, 27(7), 592-601.
- Khodaparast, N., Hays, S. A., Sloan, A. M., Hulsey, D. R., Ruiz, A., Pantoja, M., Rennaker, R. L., & Kilgard, M. P. (2013). Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. *Neurobiology of Disease*, 60, 80-88.
<https://doi.org/https://doi.org/10.1016/j.nbd.2013.08.002>
- Khodaparast, N., Kilgard, M. P., Casavant, R., Ruiz, A., Qureshi, I., Ganzer, P. D., Rennaker, R. L., & Hays, S. A. (2016). Vagus Nerve Stimulation During Rehabilitative Training Improves Forelimb Recovery After Chronic Ischemic Stroke in Rats. *Neurorehabilitation and neural repair*, 30(7), 676-684.
<https://doi.org/10.1177/1545968315616494>
- Kilavik, B. E., Zaepffel, M., Brovelli, A., MacKay, W. A., & Riehle, A. (2013). The ups and downs of beta oscillations in sensorimotor cortex. *Experimental Neurology*, 245, 15-26. <https://doi.org/https://doi.org/10.1016/j.expneurol.2012.09.014>
- Kim, H., Wright, D. L., Rhee, J., & Kim, T. (2023). C3 in the 10-20 system may not be the best target for the motor hand area. *Brain research*, 1807, 148311.
<https://doi.org/https://doi.org/10.1016/j.brainres.2023.148311>
- Kleynen, M., Braun, S. M., Bleijlevens, M. H., Lexis, M. A., Rasquin, S. M., Halfens, J., Wilson, M. R., Beurskens, A. J., & Masters, R. S. W. (2014). Using a Delphi Technique to Seek Consensus Regarding Definitions, Descriptions and Classification of Terms Related to Implicit and Explicit Forms of Motor Learning. *PLOS ONE*, 9(6), e100227.
<https://doi.org/10.1371/journal.pone.0100227>
- Kleynen, M., Braun, S. M., Rasquin, S. M. C., Bleijlevens, M. H. C., Lexis, M. A. S., Halfens, J., Wilson, M. R., Masters, R. S. W., & Beurskens, A. J. (2015). Multidisciplinary Views on Applying Explicit and Implicit Motor Learning in Practice: An International Survey. *PLOS ONE*, 10(8), e0135522.
<https://doi.org/10.1371/journal.pone.0135522>

- Kobayashi, M., Théoret, H., & Pascual-Leone, A. (2009). Suppression of ipsilateral motor cortex facilitates motor skill learning. *Eur J Neurosci*, 29(4), 833-836. <https://doi.org/10.1111/j.1460-9568.2009.06628.x>
- Koelewijn, T., van Schie, H. T., Bekkering, H., Oostenveld, R., & Jensen, O. (2008). Motor-cortical beta oscillations are modulated by correctness of observed action. *NeuroImage*, 40(2), 767-775.
- Korka, B., Will, M., Avci, I., Dukagjini, F., & Stenner, M.-P. (2023). Strategy-based motor learning decreases the post-movement β power. *Cortex*, 166, 43-58. <https://doi.org/https://doi.org/10.1016/j.cortex.2023.05.002>
- Kornysheva, K., & Diedrichsen, J. (2014). Human premotor areas parse sequences into their spatial and temporal features. *eLife*, 3, e03043. <https://doi.org/10.7554/eLife.03043>
- Kovacs, S., Peeters, R., De Ridder, D., Plazier, M., Menovsky, T., & Sunaert, S. (2011). Central Effects of Occipital Nerve Electrical Stimulation Studied by Functional Magnetic Resonance Imaging. *Neuromodulation: Technology at the Neural Interface*, 14(1), 46-57. <https://doi.org/https://doi.org/10.1111/j.1525-1403.2010.00312.x>
- Koyama, S., Tanaka, S., Tanabe, S., & Sadato, N. (2015). Dual-hemisphere transcranial direct current stimulation over primary motor cortex enhances consolidation of a ballistic thumb movement. *Neuroscience Letters*, 588, 49-53. <https://doi.org/https://doi.org/10.1016/j.neulet.2014.11.043>
- Krahl, S. E., & Clark, K. B. (2012). Vagus nerve stimulation for epilepsy: A review of central mechanisms. *Surg Neurol Int*, 3(Suppl 4), S255-259. <https://doi.org/10.4103/2152-7806.103015>
- Krakauer, J. W. (2009). Motor learning and consolidation: the case of visuomotor rotation. *Adv Exp Med Biol*, 629, 405-421. https://doi.org/10.1007/978-0-387-77064-2_21
- Krakauer, J. W., Hadjiosif, A. M., Xu, J., Wong, A. L., & Haith, A. M. (2019). Motor learning. *Compr Physiol*, 9(2), 613-663.
- Krakauer, J. W., & Shadmehr, R. (2006). Consolidation of motor memory. *Trends in Neurosciences*, 29(1), 58-64. <https://doi.org/https://doi.org/10.1016/j.tins.2005.10.003>
- Krause, V., Meier, A., Dinkelbach, L., & Pollok, B. (2016). Beta band transcranial alternating (tACS) and direct current stimulation (tDCS) applied after initial

- learning facilitate retrieval of a motor sequence. *Frontiers in Behavioral Neuroscience*, 10, 4.
- Kumar, N., Manning, T. F., & Ostry, D. J. (2019). Somatosensory cortex participates in the consolidation of human motor memory. *PLoS Biol*, 17(10), e3000469.
<https://doi.org/10.1371/journal.pbio.3000469>
- Kuntz, T., Sturm, A. P., Krause, V., Pollok, B., & Keitel, A. (2016). EP 129. The effect of tDCS over M1 prior to and during implicit motor sequence learning. *Clinical Neurophysiology*, 127(9), e295.
<https://doi.org/https://doi.org/10.1016/j.clinph.2016.05.170>
- Kuo, H. I., Paulus, W., Batsikadze, G., Jamil, A., Kuo, M. F., & Nitsche, M. A. (2017). Acute and chronic effects of noradrenergic enhancement on transcranial direct current stimulation-induced neuroplasticity in humans. *The Journal of physiology*, 595(4), 1305-1314.
- Kuo, H. I., Paulus, W., Batsikadze, G., Jamil, A., Kuo, M. F., & Nitsche, M. A. (2017). Acute and Chronic Noradrenergic Effects on Cortical Excitability in Healthy Humans. *Int J Neuropsychopharmacol*, 20(8), 634-643.
<https://doi.org/10.1093/ijnp/pyx026>
- Kuo, M.-F., Chen, P.-S., & Nitsche, M. A. (2017). The application of tDCS for the treatment of psychiatric diseases. *International Review of Psychiatry*, 29(2), 146-167.
- Lacroix, A., Proulx-Bégin, L., Hamel, R., De Beaumont, L., Bernier, P.-M., & Lepage, J.-F. (2019). Static magnetic stimulation of the primary motor cortex impairs online but not offline motor sequence learning. *Scientific Reports*, 9(1), 9886.
<https://doi.org/10.1038/s41598-019-46379-2>
- Lang, N., Nitsche, M., Paulus, W., Rothwell, J., & Lemon, R. (2004). Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Experimental brain research*, 156(4), 439-443.
- Lang, N., Nitsche, M. A., Sommer, M., Tergau, F., & Paulus, W. (2003). Chapter 28 Modulation of motor consolidation by external DC stimulation. In W. Paulus, F. Tergau, M. A. Nitsche, J. G. Rothwell, U. Ziemann, & M. Hallett (Eds.), *Supplements to Clinical Neurophysiology* (Vol. 56, pp. 277-281). Elsevier.
[https://doi.org/https://doi.org/10.1016/S1567-424X\(09\)70231-4](https://doi.org/https://doi.org/10.1016/S1567-424X(09)70231-4)

- Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, W., & Beaulieu, C. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. *NeuroImage*, 60(1), 340-352.
- Lee, K.-H., Williams, L. M., Breakspear, M., & Gordon, E. (2003). Synchronous gamma activity: a review and contribution to an integrative neuroscience model of schizophrenia. *Brain Research Reviews*, 41(1), 57-78.
- Lee, T.-H., Greening, S. G., Ueno, T., Clewett, D., Ponzio, A., Sakaki, M., & Mather, M. (2018). Arousal increases neural gain via the locus coeruleus–noradrenaline system in younger adults but not in older adults. *Nature Human Behaviour*, 2(5), 356-366. <https://doi.org/10.1038/s41562-018-0344-1>
- Lefaucheur, J.-P., Antal, A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian, F., Cotelli, M., De Ridder, D., Ferrucci, R., Langguth, B., Marangolo, P., Mylius, V., Nitsche, M. A., Padberg, F., Palm, U., Poulet, E., Priori, A., Rossi, S., Sackellmann, M., . . . Paulus, W. (2017). Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clinical Neurophysiology*, 128(1), 56-92. <https://doi.org/https://doi.org/10.1016/j.clinph.2016.10.087>
- Lefebvre, S., Dricot, L., Gradkowski, W., Laloux, P., & Vandermeeren, Y. (2012). Brain activations underlying different patterns of performance improvement during early motor skill learning. *NeuroImage*, 62(1), 290-299. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2012.04.052>
- Lefebvre, S., Laloux, P., Peeters, A., Desfontaines, P., Jamart, J., & Vandermeeren, Y. (2013). Dual-tDCS Enhances Online Motor Skill Learning and Long-Term Retention in Chronic Stroke Patients [Clinical Trial]. *Frontiers in Human Neuroscience*, 6. <https://doi.org/10.3389/fnhum.2012.00343>
- Li Voti, P., Conte, A., Suppa, A., Iezzi, E., Bologna, M., Aniello, M. S., Defazio, G., Rothwell, J. C., & Berardelli, A. (2011). Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. *Experimental brain research*, 212(1), 91-99. <https://doi.org/10.1007/s00221-011-2700-5>
- Liebe, T., Kaufmann, J., Hämmerer, D., Betts, M., & Walter, M. (2022). In vivo tractography of human locus coeruleus—relation to 7T resting state fMRI, psychological measures and single subject validity. *Molecular Psychiatry*, 1-10.
- Lissek, S., Vallana, G. S., Güntürkün, O., Dinse, H., & Tegenthoff, M. (2013). Brain Activation in Motor Sequence Learning Is Related to the Level of Native

- Cortical Excitability. *PLOS ONE*, 8(4), e61863.
<https://doi.org/10.1371/journal.pone.0061863>
- Loerwald, K. W., Buell, E. P., Borland, M. S., Rennaker, R. L., Hays, S. A., & Kilgard, M. P. (2018). Varying Stimulation Parameters to Improve Cortical Plasticity Generated by VNS-tone Pairing. *Neuroscience*, 388, 239-247.
<https://doi.org/https://doi.org/10.1016/j.neuroscience.2018.07.038>
- Louviot, S., Tyvaert, L., Maillard, L. G., Colnat-Coulbois, S., Dmochowski, J., & Koessler, L. (2022). Transcranial Electrical Stimulation generates electric fields in deep human brain structures. *Brain Stimulation*, 15(1), 1-12.
<https://doi.org/https://doi.org/10.1016/j.brs.2021.11.001>
- Luckey, A. M., McLeod, S. L., Huang, Y., Mohan, A., & Vanneste, S. (2022). Making memories last: The peripheral effect of direct current stimulation on strengthening memories. *bioRxiv*, 2022.2007.2006.498966.
<https://doi.org/10.1101/2022.07.06.498966>
- Luckey, A. M., McLeod, S. L., Mohan, A., & Vanneste, S. (2022). Potential role for peripheral nerve stimulation on learning and long-term memory: A comparison of alternating and direct current stimulations. *Brain Stimulation*, 15(3), 536-545.
<https://doi.org/https://doi.org/10.1016/j.brs.2022.03.001>
- Luckey, A. M., McLeod, S. L., Robertson, I. H., To, W. T., & Vanneste, S. (2020). Greater Occipital Nerve Stimulation Boosts Associative Memory in Older Individuals: A Randomized Trial. *Neurorehabilitation and neural repair*, 34(11), 1020-1029. <https://doi.org/10.1177/1545968320943573>
- Ludwig, M., Yi, Y.-J., Lüsebrink, F., Callaghan, M. F., Betts, M. J., Yakupov, R., Weiskopf, N., Dolan, R. J., Düzel, E., & Hämmerer, D. (2024). Functional locus coeruleus imaging to investigate an ageing noradrenergic system. *Communications Biology*, 7(1), 777. <https://doi.org/10.1038/s42003-024-06446-5>
- Luft, A. R., & Buitrago, M. M. (2005). Stages of motor skill learning. *Molecular Neurobiology*, 32(3), 205-216. <https://doi.org/10.1385/MN:32:3:205>
- Lum, J. A. G., Mills, A., Plumridge, J. M. A., Sloan, N. P., Clark, G. M., Hedenius, M., & Enticott, P. G. (2018). Transcranial direct current stimulation enhances retention of a second (but not first) order conditional visuo-motor sequence. *Brain and Cognition*, 127, 34-41.
<https://doi.org/https://doi.org/10.1016/j.bandc.2018.09.006>

- Ma, L., Narayana, S., Robin, D. A., Fox, P. T., & Xiong, J. (2011). Changes occur in resting state network of motor system during 4 weeks of motor skill learning. *NeuroImage*, 58(1), 226-233.
- Macintosh, B. J., Mraz, R., McIlroy, W. E., & Graham, S. J. (2007). Brain activity during a motor learning task: an fMRI and skin conductance study. *Hum Brain Mapp*, 28(12), 1359-1367. <https://doi.org/10.1002/hbm.20351>
- Magdoom, K. N., Subramanian, D., Chakravarthy, V. S., Ravindran, B., Amari, S.-i., & Meenakshisundaram, N. (2011). Modeling Basal Ganglia for Understanding Parkinsonian Reaching Movements. *Neural Computation*, 23(2), 477-516. https://doi.org/10.1162/NECO_a_00073
- Manaye, K. F., McIntire, D. D., Mann, D. M. A., & German, D. C. (1995). Locus coeruleus cell loss in the aging human brain: A non-random process. *Journal of Comparative Neurology*, 358(1), 79-87. <https://doi.org/https://doi.org/10.1002/cne.903580105>
- Marinelli, L., Quartarone, A., Hallett, M., Frazzitta, G., & Ghilardi, M. F. (2017). The many facets of motor learning and their relevance for Parkinson's disease. *Clin Neurophysiol*, 128(7), 1127-1141. <https://doi.org/10.1016/j.clinph.2017.03.042>
- Marrosu, F., Serra, A., Maleci, A., Puligheddu, M., Biggio, G., & Piga, M. (2003). Correlation between GABAA receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. *Epilepsy Research*, 55(1), 59-70. [https://doi.org/https://doi.org/10.1016/S0920-1211\(03\)00107-4](https://doi.org/https://doi.org/10.1016/S0920-1211(03)00107-4)
- Martin, K. A., Papadoyannis, E. S., Schiavo, J. K., Fadaei, S. S., Issa, H. A., Song, S. C., Valencia, S. O., Temiz, N. Z., McGinley, M. J., McCormick, D. A., & Froemke, R. C. (2024). Vagus nerve stimulation recruits the central cholinergic system to enhance perceptual learning. *Nature Neuroscience*. <https://doi.org/10.1038/s41593-024-01767-4>
- Mary, A., Wens, V., Op de Beeck, M., Leproult, R., De Tiège, X., & Peigneux, P. (2017). Resting-state Functional Connectivity is an Age-dependent Predictor of Motor Learning Abilities. *Cerebral Cortex*, 27(10), 4923-4932. <https://doi.org/10.1093/cercor/bhw286>
- Mather, M., Clewett, D., Sakaki, M., & Harley, C. W. (2016). Norepinephrine ignites local hotspots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behavioral and Brain Sciences*, 39, e200, Article e200. <https://doi.org/10.1017/S0140525X15000667>

- Mawase, F., Uehara, S., Bastian, A. J., & Celnik, P. (2017). Motor Learning Enhances Use-Dependent Plasticity. *J Neurosci*, 37(10), 2673-2685.
<https://doi.org/10.1523/jneurosci.3303-16.2017>
- McHuguen, S. A., Rodriguez, P. F., Kleim, J. A., Kleim, E. D., Crespo, L. M., Procaccio, V., & Cramer, S. C. (2009). BDNF Val66Met Polymorphism Influences Motor System Function in the Human Brain. *Cerebral Cortex*, 20(5), 1254-1262. <https://doi.org/10.1093/cercor/bhp189>
- Meehan, S. K., Randhawa, B., Wessel, B., & Boyd, L. A. (2011). Implicit sequence-specific motor learning after subcortical stroke is associated with increased prefrontal brain activations: An fMRI Study. *Human Brain Mapping*, 32(2), 290-303. <https://doi.org/https://doi.org/10.1002/hbm.21019>
- Meissner, S. N., Krause, V., Südmeyer, M., Hartmann, C. J., & Pollok, B. (2018). The significance of brain oscillations in motor sequence learning: Insights from Parkinson's disease. *Neuroimage Clin*, 20, 448-457.
<https://doi.org/10.1016/j.nicl.2018.08.009>
- Melcher, T., Winter, D., Hommel, B., Pfister, R., Dechent, P., & Gruber, O. (2013). The neural substrate of the ideomotor principle revisited: evidence for asymmetries in action-effect learning. *Neuroscience*, 231, 13-27.
<https://doi.org/10.1016/j.neuroscience.2012.11.035>
- Meng, H., Houston, M., Zhang, Y., & Li, S. (2024). Exploring the Prospects of Transcranial Electrical Stimulation (tES) as a Therapeutic Intervention for Post-Stroke Motor Recovery: A Narrative Review. *Brain Sciences*, 14(4), 322.
<https://www.mdpi.com/2076-3425/14/4/322>
- Meyers, E. C., Solorzano, B. R., James, J., Ganzer, P. D., Lai, E. S., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2018). Vagus Nerve Stimulation Enhances Stable Plasticity and Generalization of Stroke Recovery. *Stroke*, 49(3), 710-717.
<https://doi.org/doi:10.1161/STROKEAHA.117.019202>
- Miyaguchi, S., Inukai, Y., Matsumoto, Y., Miyashita, M., Takahashi, R., Otsuru, N., & Onishi, H. (2020). Effects on motor learning of transcranial alternating current stimulation applied over the primary motor cortex and cerebellar hemisphere. *Journal of Clinical Neuroscience*, 78, 296-300.
<https://doi.org/https://doi.org/10.1016/j.jocn.2020.05.024>
- Miyaguchi, S., Inukai, Y., Mitsumoto, S., Otsuru, N., & Onishi, H. (2022). Gamma-transcranial alternating current stimulation on the cerebellum and supplementary

- motor area improves bimanual motor skill. *Behavioural Brain Research*, 424, 113805. <https://doi.org/https://doi.org/10.1016/j.bbr.2022.113805>
- Montenegro, M. M., & Kissoon, N. R. (2023). Long term outcomes of occipital nerve stimulation. *Front Pain Res (Lausanne)*, 4, 1054764. <https://doi.org/10.3389/fpain.2023.1054764>
- Morris, L. S., Tan, A., Smith, D. A., Grehl, M., Han-Huang, K., Naidich, T. P., Charney, D. S., Balchandani, P., Kundu, P., & Murrough, J. W. (2020). Sub-millimeter variation in human locus coeruleus is associated with dimensional measures of psychopathology: An in vivo ultra-high field 7-Tesla MRI study. *NeuroImage: Clinical*, 25, 102148. <https://doi.org/https://doi.org/10.1016/j.nicl.2019.102148>
- Mosayebi Samani, M., Agboada, D., Jamil, A., Kuo, M.-F., & Nitsche, M. A. (2019). Titrating the neuroplastic effects of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex. *Cortex*, 119, 350-361. <https://doi.org/https://doi.org/10.1016/j.cortex.2019.04.016>
- Mottaz, A., Savic, B., Allaman, L., & Guggisberg, A. G. (2024). Neural correlates of motor learning: Network communication versus local oscillations. *Network Neuroscience*, 1-20. https://doi.org/10.1162/netn_a_00374
- Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., Boroojerdi, B., Poewe, W., & Hallett, M. (2002). Early consolidation in human primary motor cortex. *Nature*, 415(6872), 640-644. <https://doi.org/10.1038/nature712>
- Müller, R.-A., Kleinhans, N., Pierce, K., Kemmotsu, N., & Courchesne, E. (2002). Functional MRI of motor sequence acquisition: effects of learning stage and performance. *Cognitive Brain Research*, 14(2), 277-293. [https://doi.org/https://doi.org/10.1016/S0926-6410\(02\)00131-3](https://doi.org/https://doi.org/10.1016/S0926-6410(02)00131-3)
- Murphy, P. R., O'connell, R. G., O'sullivan, M., Robertson, I. H., & Balsters, J. H. (2014). Pupil diameter covaries with BOLD activity in human locus coeruleus. *Human Brain Mapping*, 35(8), 4140-4154.
- Nachev, P., Wydell, H., O'Neill, K., Husain, M., & Kennard, C. (2007). The role of the pre-supplementary motor area in the control of action. *NeuroImage*, 36, T155-T163. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2007.03.034>
- Nakazono, H., Ogata, K., Takeda, A., Yamada, E., Oka, S., & Tobimatsu, S. (2021). A specific phase of transcranial alternating current stimulation at the β frequency

- boosts repetitive paired-pulse TMS-induced plasticity. *Scientific Reports*, 11(1), 13179. <https://doi.org/10.1038/s41598-021-92768-x>
- Naro, A., Bramanti, A., Leo, A., Manuli, A., Sciarrone, F., Russo, M., Bramanti, P., & Calabrò, R. S. (2017). Effects of cerebellar transcranial alternating current stimulation on motor cortex excitability and motor function. *Brain Structure and Function*, 222(6), 2891-2906.
- Naro, A., Leo, A., Russo, M., Cannavò, A., Milardi, D., Bramanti, P., & Calabrò, R. S. (2016). Does Transcranial Alternating Current Stimulation Induce Cerebellum Plasticity? Feasibility, Safety and Efficacy of a Novel Electrophysiological Approach. *Brain Stimulation*, 9(3), 388-395.
<https://doi.org/https://doi.org/10.1016/j.brs.2016.02.005>
- Neuling, T., Ruhnau, P., Weisz, N., Herrmann, C. S., & Demarchi, G. (2017). Faith and oscillations recovered: On analyzing EEG/MEG signals during tACS. *NeuroImage*, 147, 960-963.
<https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.11.022>
- Nguyen, K. P., Zhou, W., McKenna, E., Colucci-Chang, K., Bray, L. C. J., Hosseini, E. A., Alhussein, L., Rezazad, M., & Joiner, W. M. (2019). The 24-h savings of adaptation to novel movement dynamics initially reflects the recall of previous performance. *Journal of Neurophysiology*, 122(3), 933-946.
<https://doi.org/10.1152/jn.00569.2018>
- Nitsche, M. A., Kuo, M.-F., Karrasch, R., Wächter, B., Liebetanz, D., & Paulus, W. (2009). Serotonin affects transcranial direct current–induced neuroplasticity in humans. *Biological psychiatry*, 66(5), 503-508.
- Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol*, 527 Pt 3(Pt 3), 633-639. <https://doi.org/10.1111/j.1469-7793.2000.t01-1-00633.x>
- Nitsche, M. A., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., & Tergau, F. (2003). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of cognitive neuroscience*, 15(4), 619-626.
- Noury, N., Hipp, J. F., & Siegel, M. (2016). Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. *NeuroImage*, 140, 99-109.
<https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.03.065>

- Noury, N., & Siegel, M. (2018). Analyzing EEG and MEG signals recorded during tES, a reply. *NeuroImage*, 167, 53-61.
<https://doi.org/https://doi.org/10.1016/j.neuroimage.2017.11.023>
- Nowak, M., Hinson, E., Van Ede, F., Pogosyan, A., Guerra, A., Quinn, A., Brown, P., & Stagg, C. J. (2017). Driving human motor cortical oscillations leads to behaviorally relevant changes in local GABAA inhibition: a tACS-TMS study. *Journal of Neuroscience*, 37(17), 4481-4492.
- Nowak, M., Zich, C., & Stagg, C. J. (2018). Motor Cortical Gamma Oscillations: What Have We Learnt and Where Are We Headed? *Curr Behav Neurosci Rep*, 5(2), 136-142. <https://doi.org/10.1007/s40473-018-0151-z>
- Oh, E., Park, J., Youn, J., & Jang, W. (2022). Anodal Transcranial Direct Current Stimulation Could Modulate Cortical Excitability and the Central Cholinergic System in Akinetic Rigid-Type Parkinson's Disease: Pilot Study. *Frontiers in Neurology*, 13.
- Oishi, K., Toma, K., Bagarinao, E. T., Matsuo, K., Nakai, T., Chihara, K., & Fukuyama, H. (2005). Activation of the precuneus is related to reduced reaction time in serial reaction time tasks. *Neuroscience Research*, 52(1), 37-45.
<https://doi.org/https://doi.org/10.1016/j.neures.2005.01.008>
- Olson, I. R., Rao, H., Moore, K. S., Wang, J., Detre, J. A., & Aguirre, G. K. (2006). Using perfusion fMRI to measure continuous changes in neural activity with learning. *Brain and Cognition*, 60(3), 262-271.
- Orban, P., Peigneux, P., Lungu, O., Albouy, G., Breton, E., Laberrenne, F., Benali, H., Maquet, P., & Doyon, J. (2010). The multifaceted nature of the relationship between performance and brain activity in motor sequence learning. *NeuroImage*, 49(1), 694-702. <https://doi.org/10.1016/j.neuroimage.2009.08.055>
- Orban, P., Peigneux, P., Lungu, O., Debas, K., Barakat, M., Bellec, P., Benali, H., Maquet, P., & Doyon, J. (2011). Functional neuroanatomy associated with the expression of distinct movement kinematics in motor sequence learning. *Neuroscience*, 179, 94-103.
<https://doi.org/https://doi.org/10.1016/j.neuroscience.2011.01.040>
- Ostry, D. J., Darainy, M., Mattar, A. A., Wong, J., & Gribble, P. L. (2010). Somatosensory plasticity and motor learning. *J Neurosci*, 30(15), 5384-5393.
<https://doi.org/10.1523/jneurosci.4571-09.2010>

- Otero, M., Prado-Gutiérrez, P., Weinstein, A., Escobar, M. J., & El-Deredy, W. (2020). Persistence of EEG Alpha Entrainment Depends on Stimulus Phase at Offset. *Front Hum Neurosci*, 14, 139. <https://doi.org/10.3389/fnhum.2020.00139>
- Pagge, C., Caballero-Insaurriaga, J., Oliviero, A., Foffani, G., & Ammann, C. (2024). Transcranial static magnetic field stimulation of the supplementary motor area decreases corticospinal excitability in the motor cortex: a pilot study. *Scientific Reports*, 14(1), 6597. <https://doi.org/10.1038/s41598-024-57030-0>
- Pammi, V. S., Miyapuram, K. P., Ahmed, Samejima, K., Bapi, R. S., & Doya, K. (2012). Changing the structure of complex visuo-motor sequences selectively activates the fronto-parietal network. *NeuroImage*, 59(2), 1180-1189. <https://doi.org/10.1016/j.neuroimage.2011.08.006>
- Papale, A. E., & Hooks, B. M. (2018). Circuit changes in motor cortex during motor skill learning. *Neuroscience*, 368, 283-297. <https://doi.org/10.1016/j.neuroscience.2017.09.010>
- Patel, H. J., Romanzetti, S., Pellicano, A., Reetz, K., & Binkofski, F. (2018). PB11. Long term GABA alterations induced by anodal Transcranial Direct Current Stimulation (tDCS) in motor cortex: A proton magnetic resonance spectroscopy study. *Clinical Neurophysiology*, 129(8), e59-e60. <https://doi.org/https://doi.org/10.1016/j.clinph.2018.04.636>
- Penhune, V. B., & Doyon, J. (2005). Cerebellum and M1 interaction during early learning of timed motor sequences. *NeuroImage*, 26(3), 801-812. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2005.02.041>
- Perez, M. A., Wise, S. P., Willingham, D. T., & Cohen, L. G. (2007). Neurophysiological Mechanisms Involved in Transfer of Procedural Knowledge. *The Journal of Neuroscience*, 27(5), 1045-1053. <https://doi.org/10.1523/jneurosci.4128-06.2007>
- Peterson, R. A., & Merunka, D. R. (2014). Convenience samples of college students and research reproducibility. *Journal of Business Research*, 67(5), 1035-1041. <https://doi.org/https://doi.org/10.1016/j.jbusres.2013.08.010>
- Pirondini, E., Coscia, M., Minguillon, J., Millán, J. d. R., Van De Ville, D., & Micera, S. (2017). EEG topographies provide subject-specific correlates of motor control. *Scientific Reports*, 7(1), 13229. <https://doi.org/10.1038/s41598-017-13482-1>

- Pixa, N. H., & Pollok, B. (2018). Effects of tDCS on Bimanual Motor Skills: A Brief Review [Mini Review]. *Frontiers in Behavioral Neuroscience*, 12.
<https://doi.org/10.3389/fnbeh.2018.00063>
- Plewnia, C., Hoppe, J., Cohen, L. G., & Gerloff, C. (2004). Improved motor skill acquisition after selective stimulation of central norepinephrine. *Neurology*, 62(11), 2124-2126. <https://doi.org/doi:10.1212/01.WNL.0000128041.92710.17>
- Pollok, B., Boysen, A.-C., & Krause, V. (2015a). The effect of transcranial alternating current stimulation (tACS) at alpha and beta frequency on motor learning. *Behavioural Brain Research*, 293, 234-240.
<https://doi.org/https://doi.org/10.1016/j.bbr.2015.07.049>
- Pollok, B., Boysen, A., & Krause, V. (2015b). The effect of transcranial alternating current stimulation (tACS) at alpha and beta frequency on motor learning. *Behavioural Brain Research*, 293, 234-240.
- Pollok, B., Keitel, A., Foerster, M., Moshiri, G., Otto, K., & Krause, V. (2020). The posterior parietal cortex mediates early offline-rather than online-motor sequence learning. *Neuropsychologia*, 146, 107555.
<https://doi.org/https://doi.org/10.1016/j.neuropsychologia.2020.107555>
- Pollok, B., Latz, D., Krause, V., Butz, M., & Schnitzler, A. (2014). Changes of motor-cortical oscillations associated with motor learning. *Neuroscience*, 275, 47-53.
- Porrill, J., & Dean, P. (2007). Recurrent Cerebellar Loops Simplify Adaptive Control of Redundant and Nonlinear Motor Systems. *Neural Computation*, 19(1), 170-193.
<https://doi.org/10.1162/neco.2007.19.1.170>
- Porter, B. A., Khodaparast, N., Fayyaz, T., Cheung, R. J., Ahmed, S. S., Vrana, W. A., Rennaker, R. L., II, & Kilgard, M. P. (2011). Repeatedly Pairing Vagus Nerve Stimulation with a Movement Reorganizes Primary Motor Cortex. *Cerebral Cortex*, 22(10), 2365-2374. <https://doi.org/10.1093/cercor/bhr316>
- Pruitt, D. T., Danaphongse, T. T., Lutchman, M., Patel, N., Reddy, P., Wang, V., Parashar, A., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2021). Optimizing Dosing of Vagus Nerve Stimulation for Stroke Recovery. *Translational Stroke Research*, 12(1), 65-71. <https://doi.org/10.1007/s12975-020-00829-6>
- Rahman, A., Reato, D., Arlotti, M., Gasca, F., Datta, A., Parra, L. C., & Bikson, M. (2013). Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *The Journal of physiology*, 591(10), 2563-2578.

- Ramanathan, D., Conner, J. M., & H. Tuszynski, M. (2006). A form of motor cortical plasticity that correlates with recovery of function after brain injury. *Proceedings of the National Academy of Sciences*, 103(30), 11370-11375. <https://doi.org/doi:10.1073/pnas.0601065103>
- Rasmusson, D. D. (2000). The role of acetylcholine in cortical synaptic plasticity. *Behavioural Brain Research*, 115(2), 205-218. [https://doi.org/https://doi.org/10.1016/S0166-4328\(00\)00259-X](https://doi.org/https://doi.org/10.1016/S0166-4328(00)00259-X)
- Rau, C., Plewnia, C., Hummel, F., & Gerloff, C. (2003). Event-related desynchronization and excitability of the ipsilateral motor cortex during simple self-paced finger movements. *Clinical Neurophysiology*, 114(10), 1819-1826. [https://doi.org/https://doi.org/10.1016/S1388-2457\(03\)00174-3](https://doi.org/https://doi.org/10.1016/S1388-2457(03)00174-3)
- Reed, T., & Cohen Kadosh, R. (2018). Transcranial electrical stimulation (tES) mechanisms and its effects on cortical excitability and connectivity. *J Inherit Metab Dis*, 41(6), 1123-1130. <https://doi.org/10.1007/s10545-018-0181-4>
- Reimer, J., McGinley, M. J., Liu, Y., Rodenkirch, C., Wang, Q., McCormick, D. A., & Tolia, A. S. (2016). Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. *Nature communications*, 7(1), 13289.
- Reis, J., & Fritsch, B. (2011). Modulation of motor performance and motor learning by transcranial direct current stimulation. *Current opinion in neurology*, 24(6), 590-596.
- Rémy, F., Wenderoth, N., Lipkens, K., & Swinnen, S. P. (2008). Acquisition of a new bimanual coordination pattern modulates the cerebral activations elicited by an intrinsic pattern: an fMRI study. *Cortex*, 44(5), 482-493.
- Richards, L. G., Stewart, K. C., Woodbury, M. L., Senesac, C., & Cauraugh, J. H. (2008). Movement-dependent stroke recovery: A systematic review and meta-analysis of TMS and fMRI evidence. *Neuropsychologia*, 46(1), 3-11. <https://doi.org/https://doi.org/10.1016/j.neuropsychologia.2007.08.013>
- Rieth, C. A., Cai, D. J., McDevitt, E. A., & Mednick, S. C. (2010). The role of sleep and practice in implicit and explicit motor learning. *Behavioural Brain Research*, 214(2), 470-474. <https://doi.org/https://doi.org/10.1016/j.bbr.2010.05.052>
- Robertson, E. M. (2007). The serial reaction time task: implicit motor skill learning? *J Neurosci*, 27(38), 10073-10075. <https://doi.org/10.1523/jneurosci.2747-07.2007>

- Robertson, E. M., Pascual-Leone, A., & Miall, R. C. (2004). Current concepts in procedural consolidation. *Nature Reviews Neuroscience*, 5(7), 576-582. <https://doi.org/10.1038/nrn1426>
- Roche, N., Geiger, M., & Bussel, B. (2015). Mechanisms underlying transcranial direct current stimulation in rehabilitation. *Annals of physical and rehabilitation medicine*, 58(4), 214-219.
- Rommelfanger, K. S., Edwards, G. L., Freeman, K. G., Liles, L. C., Miller, G. W., & Weinshenker, D. (2007). Norepinephrine loss produces more profound motor deficits than MPTP treatment in mice. *Proceedings of the National Academy of Sciences*, 104(34), 13804-13809. <https://doi.org/doi:10.1073/pnas.0702753104>
- Roshchupkina, L., Stee, W., & Peigneux, P. (2020). Beta-tACS does not impact the dynamics of motor memory consolidation. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 13(6), 1489-1490.
- Roshchupkina, L., Wens, V., Coquelet, N., de Tiege, X., & Peigneux, P. (2022). Resting state fast brain dynamics predict interindividual variability in motor performance. *Sci Rep*, 12(1), 5340. <https://doi.org/10.1038/s41598-022-08767-z>
- Rossi, S., Antal, A., Bestmann, S., Bikson, M., Brewer, C., Brockmöller, J., Carpenter, L. L., Cincotta, M., Chen, R., Daskalakis, J. D., Di Lazzaro, V., Fox, M. D., George, M. S., Gilbert, D., Kimiskidis, V. K., Koch, G., Ilmoniemi, R. J., Lefaucheur, J. P., Leocani, L., . . . Hallett, M. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clinical Neurophysiology*, 132(1), 269-306. <https://doi.org/https://doi.org/10.1016/j.clinph.2020.10.003>
- Röttger, E., Zhao, F., Gaschler, R., & Haider, H. (2021). Why Does Dual-Tasking Hamper Implicit Sequence Learning? *J Cogn*, 4(1), 1. <https://doi.org/10.5334/joc.136>
- Rumpf, J.-J., Barbu, A., Fricke, C., Wegscheider, M., & Classen, J. (2019). Posttraining alpha transcranial alternating current stimulation impairs motor consolidation in elderly people. *Neural Plasticity*, 2019.
- Sacco, K., Cauda, F., D'Agata, F., Mate, D., Duca, S., & Geminiani, G. (2009). Reorganization and enhanced functional connectivity of motor areas in repetitive ankle movements after training in locomotor attention. *Brain Res*, 1297, 124-134. <https://doi.org/10.1016/j.brainres.2009.08.049>

- Sales, A. C., Friston, K. J., Jones, M. W., Pickering, A. E., & Moran, R. J. (2019). Locus Coeruleus tracking of prediction errors optimises cognitive flexibility: An Active Inference model. *PLoS computational biology*, 15(1), e1006267.
- Salo, T. a., Yarkoni, T. a., Nichols, T. E. a., Poline, J.-B. a., Kent, J. D. a., Gorgolewski, K. J. a., Glerean, E. a., Bottenhorn, K. L. a., Bilgel, M. a., Wright, J. a., Reeders, P. a., Kimbler, A. a., Nielson, D. N. a., Yanes, J. A. a., Pérez, A. a., Oudyk, K. M. a., Jarecka, D. a., Enge, A. a., Peraza, J. A. a., . . . Laird, A. R. (2023). *neurostuff/NiMARE: 0.0.13*. In Zenodo.
- Samaci, A., Ehsani, F., Zoghi, M., Hafez Yosephi, M., & Jaberzadeh, S. (2017). Online and offline effects of cerebellar transcranial direct current stimulation on motor learning in healthy older adults: a randomized double-blind sham-controlled study. *Eur J Neurosci*, 45(9), 1177-1185. <https://doi.org/10.1111/ejn.13559>
- Sami, S., & Miall, R. (2013). Graph network analysis of immediate motor-learning induced changes in resting state BOLD [Original Research]. *Frontiers in Human Neuroscience*, 7. <https://doi.org/10.3389/fnhum.2013.00166>
- Sasaki, R., Miyaguchi, S., Kotan, S., Kojima, S., Kirimoto, H., & Onishi, H. (2016). Modulation of cortical inhibitory circuits after cathodal transcranial direct current stimulation over the primary motor cortex. *Frontiers in Human Neuroscience*, 10, 30.
- Schlund, M. W., Rosales-Ruiz, J., Vaidya, M., Glenn, S. S., & Staff, D. (2008). Experience-dependent plasticity: differential changes in activation associated with repeated reinforcement. *Neuroscience*, 155(1), 17-23. <https://doi.org/10.1016/j.neuroscience.2008.04.076>
- Schwarb, H., & Schumacher, E. H. (2012). Generalized lessons about sequence learning from the study of the serial reaction time task. *Adv Cogn Psychol*, 8(2), 165-178. <https://doi.org/10.2478/v10053-008-0113-1>
- Sears, D. O. (1986). College sophomores in the laboratory: Influences of a narrow data base on social psychology's view of human nature. *Journal of Personality and Social Psychology*, 51(3), 515-530. <https://doi.org/10.1037/0022-3514.51.3.515>
- Sehatpour, P., Kreither, J., Lopez-Calderon, J., Shastry, A. M., De Baun, H. M., Martinez, A., & Javitt, D. C. (2023). Network-level mechanisms underlying effects of transcranial direct current stimulation (tDCS) on visuomotor learning in schizophrenia. *Translational Psychiatry*, 13(1), 360. <https://doi.org/10.1038/s41398-023-02656-3>

- Seidler, R. D., Bo, J., & Anguera, J. A. (2012). Neurocognitive contributions to motor skill learning: the role of working memory. *J Mot Behav*, 44(6), 445-453.
<https://doi.org/10.1080/00222895.2012.672348>
- Seminck, N., Khatoun, A., Kerstens, S., Nuttin, B., & Mc Laughlin, M. (2024). External stimulation of the trigeminal nerve causes pupil dilation in healthy volunteers, suggesting locus coeruleus modulation. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*, 17(3), 678-680.
<https://doi.org/10.1016/j.brs.2024.05.008>
- Shadmehr, R. (2018). Motor Learning: A Cortical System for Adaptive Motor Control. *Current Biology*, 28(14), R793-R795.
<https://doi.org/https://doi.org/10.1016/j.cub.2018.05.071>
- Shadmehr, R., & Brashers-Krug, T. (1997). Functional Stages in the Formation of Human Long-Term Motor Memory. *The Journal of Neuroscience*, 17(1), 409-419. <https://doi.org/10.1523/jneurosci.17-01-00409.1997>
- Shadmehr, R., & Krakauer, J. W. (2008). A computational neuroanatomy for motor control. *Experimental brain research*, 185(3), 359-381.
<https://doi.org/10.1007/s00221-008-1280-5>
- Shadmehr, R., Smith, M. A., & Krakauer, J. W. (2010). Error correction, sensory prediction, and adaptation in motor control. *Annual review of neuroscience*, 33(1), 89-108.
- Sharififar, S., Shuster, J. J., & Bishop, M. D. (2018). Adding electrical stimulation during standard rehabilitation after stroke to improve motor function. A systematic review and meta-analysis. *Annals of physical and rehabilitation medicine*, 61(5), 339-344.
<https://doi.org/https://doi.org/10.1016/j.rehab.2018.06.005>
- Shmuelof, L., & Krakauer, John W. (2011). Are We Ready for a Natural History of Motor Learning? *Neuron*, 72(3), 469-476.
<https://doi.org/https://doi.org/10.1016/j.neuron.2011.10.017>
- Sidarta, A., Vahdat, S., Bernardi, N. F., & Ostry, D. J. (2016). Somatic and reinforcement-based plasticity in the initial stages of human motor learning. *Journal of Neuroscience*, 36(46), 11682-11692.
- Spampinato, D., & Celnik, P. (2021). Multiple Motor Learning Processes in Humans: Defining Their Neurophysiological Bases. *Neuroscientist*, 27(3), 246-267.
<https://doi.org/10.1177/1073858420939552>

- Spampinato, D. A., Satar, Z., & Rothwell, J. C. (2019). Combining reward and M1 transcranial direct current stimulation enhances the retention of newly learnt sensorimotor mappings. *Brain Stimulation*, 12(5), 1205-1212.
- Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011). The role of GABA in human motor learning. *Curr Biol*, 21(6), 480-484.
<https://doi.org/10.1016/j.cub.2011.01.069>
- Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., Morris, P. G., Matthews, P. M., & Johansen-Berg, H. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J Neurosci*, 29(16), 5202-5206. <https://doi.org/10.1523/jneurosci.4432-08.2009>
- Struber, L., Baumont, M., Barraud, P.-A., Nougier, V., & Cignetti, F. (2021). Brain oscillatory correlates of visuomotor adaptive learning. *NeuroImage*, 245, 118645. <https://doi.org/https://doi.org/10.1016/j.neuroimage.2021.118645>
- Tadel, F., Baillet, S., Mosher, J. C., Pantazis, D., & Leahy, R. M. (2011). Brainstorm: A User-Friendly Application for MEG/EEG Analysis. *Computational Intelligence and Neuroscience*, 2011, 879716. <https://doi.org/10.1155/2011/879716>
- Takemi, M., Masakado, Y., Liu, M., & Ushiba, J. (2013). Event-related desynchronization reflects downregulation of intracortical inhibition in human primary motor cortex. *Journal of Neurophysiology*, 110(5), 1158-1166.
<https://doi.org/10.1152/jn.01092.2012>
- Takeuchi, N., & Izumi, S. I. (2021). Motor Learning Based on Oscillatory Brain Activity Using Transcranial Alternating Current Stimulation: A Review. *Brain Sci*, 11(8). <https://doi.org/10.3390/brainsci11081095>
- Tamaki, M., Huang, T.-R., Yotsumoto, Y., Hämäläinen, M., Lin, F.-H., José E. Náñez, S., Watanabe, T., & Sasaki, Y. (2013). Enhanced Spontaneous Oscillations in the Supplementary Motor Area Are Associated with Sleep-Dependent Offline Learning of Finger-Tapping Motor-Sequence Task. *The Journal of Neuroscience*, 33(34), 13894-13902. <https://doi.org/10.1523/jneurosci.1198-13.2013>
- Tamás Kincses, Z., Johansen-Berg, H., Tomassini, V., Bosnell, R., Matthews, P. M., & Beckmann, C. F. (2008). Model-free characterization of brain functional networks for motor sequence learning using fMRI. *NeuroImage*, 39(4), 1950-1958. <https://doi.org/10.1016/j.neuroimage.2007.09.070>

- Tan, H., Jenkinson, N., & Brown, P. (2014). Dynamic neural correlates of motor error monitoring and adaptation during trial-to-trial learning. *J Neurosci*, 34(16), 5678-5688. <https://doi.org/10.1523/jneurosci.4739-13.2014>
- Tanaka, S., Honda, M., Hanakawa, T., & Cohen, L. G. (2009). Differential Contribution of the Supplementary Motor Area to Stabilization of a Procedural Motor Skill Acquired through Different Practice Schedules. *Cerebral Cortex*, 20(9), 2114-2121. <https://doi.org/10.1093/cercor/bhp276>
- Tani, G., Corrêa, U. C., Basso, L., Benda, R. N., Ugrinowitsch, H., & Choshi, K. (2014). An adaptive process model of motor learning: insights for the teaching of motor skills. *Nonlinear Dynamics Psychol Life Sci*, 18(1), 47-65.
- Thach, W. T. (1998). What is the role of the cerebellum in motor learning and cognition? *Trends in Cognitive Sciences*, 2(9), 331-337. [https://doi.org/10.1016/S1364-6613\(98\)01223-6](https://doi.org/10.1016/S1364-6613(98)01223-6)
- Titone, S., Samogin, J., Peigneux, P., Swinnen, S., Mantini, D., & Albouy, G. (2022). Connectivity in Large-Scale Resting-State Brain Networks Is Related to Motor Learning: A High-Density EEG Study. *Brain Sci*, 12(5). <https://doi.org/10.3390/brainsci12050530>
- Tomassini, V., Jbabdi, S., Kincses, Z. T., Bosnell, R., Douaud, G., Pozzilli, C., Matthews, P. M., & Johansen-Berg, H. (2011). Structural and functional bases for individual differences in motor learning. *Human Brain Mapping*, 32(3), 494-508.
- Tracy, J. I., Faro, S. S., Mohammed, F., Pinus, A., Christensen, H., & Burkland, D. (2001). A comparison of 'Early' and 'Late' stage brain activation during brief practice of a simple motor task. *Brain Res Cogn Brain Res*, 10(3), 303-316. [https://doi.org/10.1016/s0926-6410\(00\)00045-8](https://doi.org/10.1016/s0926-6410(00)00045-8)
- Truong, C., Ruffino, C., Gaveau, J., White, O., Hilt, P. M., & Papaxanthis, C. (2023). Time of day and sleep effects on motor acquisition and consolidation. *npj Science of Learning*, 8(1), 30. <https://doi.org/10.1038/s41539-023-00176-9>
- Tseng, C.-T., Gaulding, S. J., Dancel, C. L. E., & Thorn, C. A. (2021). Local activation of $\alpha 2$ adrenergic receptors is required for vagus nerve stimulation induced motor cortical plasticity. *Scientific Reports*, 11(1), 21645. <https://doi.org/10.1038/s41598-021-00976-2>
- Tseng, C. T., Welch, H. F., Gi, A. L., Kang, E. M., Mamidi, T., Pydimarri, S., Ramesh, K., Sandoval, A., Ploski, J. E., & Thorn, C. A. (2024). Frequency Specific

- Optogenetic Stimulation of the Locus Coeruleus Induces Task-Relevant Plasticity in the Motor Cortex. *J Neurosci*, 44(7).
<https://doi.org/10.1523/jneurosci.1528-23.2023>
- Tung, K. C., Uh, J., Mao, D., Xu, F., Xiao, G., & Lu, H. (2013). Alterations in resting functional connectivity due to recent motor task. *NeuroImage*, 78, 316-324.
<https://doi.org/10.1016/j.neuroimage.2013.04.006>
- Tunovic, S., Press, D. Z., & Robertson, E. M. (2014). A Physiological Signal That Prevents Motor Skill Improvements during Consolidation. *The Journal of Neuroscience*, 34(15), 5302-5310. <https://doi.org/10.1523/jneurosci.3497-13.2014>
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., & Joliot, M. (2002). Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. *NeuroImage*, 15(1), 273-289.
<https://doi.org/https://doi.org/10.1006/nimg.2001.0978>
- Tzvi, E., Loens, S., & Donchin, O. (2022). Mini-review: The Role of the Cerebellum in Visuomotor Adaptation. *The Cerebellum*, 21(2), 306-313.
<https://doi.org/10.1007/s12311-021-01281-4>
- Tzvi, E., Münte, T. F., & Krämer, U. M. (2014). Delineating the cortico-striatal-cerebellar network in implicit motor sequence learning. *NeuroImage*, 94, 222-230.
- Unal, G., Ficek, B., Webster, K., Shahabuddin, S., Truong, D., Hampstead, B., Bikson, M., & Tsapkini, K. (2020). Impact of brain atrophy on tDCS and HD-tDCS current flow: a modeling study in three variants of primary progressive aphasia. *Neurological Sciences*, 41(7), 1781-1789. <https://doi.org/10.1007/s10072-019-04229-z>
- Ura, H., Sugaya, Y., Ohata, H., Takumi, I., Sadamoto, K., Shibasaki, T., & Maru, E. (2013). Vagus nerve stimulation induced long-lasting enhancement of synaptic transmission and decreased granule cell discharge in the hippocampal dentate gyrus of urethane-anesthetized rats. *Brain research*, 1492, 63-71.
- Vahdat, S., Darainy, M., Milner, T. E., & Ostry, D. J. (2011). Functionally specific changes in resting-state sensorimotor networks after motor learning. *Journal of Neuroscience*, 31(47), 16907-16915.

- van Boekholdt, L., Kerstens, S., Khatoun, A., Asamoah, B., & Mc Laughlin, M. (2021). tDCS peripheral nerve stimulation: a neglected mode of action? *Molecular Psychiatry*, 26(2), 456-461.
- Van den Berg, N., Al-Kuwatli, J., Paulin, J., Ray, L., Owen, A., & Fogel, S. (2019). Sleep preferentially enhances memory for a cognitive strategy but not the implicit motor skills used to acquire it. *Neurobiology of Learning and Memory*, 161, 135-142.
- Vanneste, S., & De Ridder, D. (2011). Bifrontal transcranial direct current stimulation modulates tinnitus intensity and tinnitus-distress-related brain activity. *European Journal of Neuroscience*, 34(4), 605-614.
- Vanneste, S., Mohan, A., Yoo, H., Huang, Y., Luckey, A., McLeod, S., Tabet, M., Souza, R., McIntyre, C., Chapman, S., Robertson, I., & To, W. T. (2020). The peripheral effect of direct current stimulation on brain circuits involving memory. *Science Advances*, 6. <https://doi.org/10.1126/sciadv.aax9538>
- Vazey, E. M., Moorman, D. E., & Aston-Jones, G. (2018). Phasic locus coeruleus activity regulates cortical encoding of salience information. *Proceedings of the National Academy of Sciences*, 115(40), E9439-E9448. <https://doi.org/doi:10.1073/pnas.1803716115>
- Vékony, T., Ambrus, G. G., Janacsek, K., & Nemeth, D. (2022). Cautious or causal? Key implicit sequence learning paradigms should not be overlooked when assessing the role of DLPFC (Commentary on Prutean et al.). *Cortex*, 148, 222-226. <https://doi.org/https://doi.org/10.1016/j.cortex.2021.10.001>
- Verhoeven, F. M., & Newell, K. M. (2018). Unifying practice schedules in the timescales of motor learning and performance. *Human Movement Science*, 59, 153-169. <https://doi.org/https://doi.org/10.1016/j.humov.2018.04.004>
- Vidoni, E. D., Acerra, N. E., Dao, E., Meehan, S. K., & Boyd, L. A. (2010). Role of the primary somatosensory cortex in motor learning: An rTMS study. *Neurobiology of Learning and Memory*, 93(4), 532-539. <https://doi.org/https://doi.org/10.1016/j.nlm.2010.01.011>
- Voelcker-Rehage, C. (2008). Motor-skill learning in older adults—a review of studies on age-related differences. *European Review of Aging and Physical Activity*, 5(1), 5-16. <https://doi.org/10.1007/s11556-008-0030-9>
- Vollmann, H., Conde, V., Sewerin, S., Taubert, M., Sehm, B., Witte, O. W., Villringer, A., & Ragert, P. (2013). Anodal transcranial direct current stimulation (tDCS)

- over supplementary motor area (SMA) but not pre-SMA promotes short-term visuomotor learning. *Brain Stimulation*, 6(2), 101-107.
<https://doi.org/https://doi.org/10.1016/j.brs.2012.03.018>
- Vöröslakos, M., Takeuchi, Y., Brinyiczki, K., Zombori, T., Oliva, A., Fernández-Ruiz, A., Kozák, G., Kincses, Z. T., Iványi, B., & Buzsáki, G. (2018). Direct effects of transcranial electric stimulation on brain circuits in rats and humans. *Nature communications*, 9(1), 1-17.
- Vosskuhl, J., Huster, R. J., & Herrmann, C. S. (2016). BOLD signal effects of transcranial alternating current stimulation (tACS) in the alpha range: A concurrent tACS-fMRI study. *NeuroImage*, 140, 118-125.
<https://doi.org/10.1016/j.neuroimage.2015.10.003>
- Wach, C., Krause, V., Moliadze, V., Paulus, W., Schnitzler, A., & Pollok, B. (2013). Effects of 10Hz and 20Hz transcranial alternating current stimulation (tACS) on motor functions and motor cortical excitability. *Behavioural Brain Research*, 241, 1-6. <https://doi.org/https://doi.org/10.1016/j.bbr.2012.11.038>
- Wali, M. (2020). Role of the somatosensory cortex in motor memory consolidation. *Journal of Neurophysiology*, 124(3), 648-651.
<https://doi.org/10.1152/jn.00770.2019>
- Wang, J., & Sainburg, R. L. (2004). Interlimb Transfer of Novel Inertial Dynamics Is Asymmetrical. *Journal of Neurophysiology*, 92(1), 349-360.
<https://doi.org/10.1152/jn.00960.2003>
- Wang, X., Casadio, M., Weber, K. A., 2nd, Mussa-Ivaldi, F. A., & Parrish, T. B. (2014). White matter microstructure changes induced by motor skill learning utilizing a body machine interface. *NeuroImage*, 88, 32-40.
<https://doi.org/10.1016/j.neuroimage.2013.10.066>
- Watanabe, R., Watanabe, S., Kuruma, H., Murakami, Y., Seno, A., & Matsuda, T. (2011). Neural activation during imitation of movements presented from four different perspectives: a functional magnetic resonance imaging study. *Neurosci Lett*, 503(2), 100-104. <https://doi.org/10.1016/j.neulet.2011.08.016>
- Weaver, J. (2015). Motor Learning Unfolds over Different Timescales in Distinct Neural Systems. *PLoS Biol*, 13(12), e1002313.
<https://doi.org/10.1371/journal.pbio.1002313>

- Wiltshire, C. E. E., & Watkins, K. E. (2020). Failure of tDCS to modulate motor excitability and speech motor learning. *Neuropsychologia*, 146, 107568. <https://doi.org/https://doi.org/10.1016/j.neuropsychologia.2020.107568>
- Wischnewski, M., Engelhardt, M., Salehinejad, M. A., Schutter, D. J. L. G., Kuo, M. F., & Nitsche, M. A. (2019). NMDA Receptor-Mediated Motor Cortex Plasticity After 20 Hz Transcranial Alternating Current Stimulation. *Cerebral Cortex*, 29(7), 2924-2931. <https://doi.org/10.1093/cercor/bhy160>
- Wischnewski, M., Schutter, D. J. L. G., & Nitsche, M. A. (2019). Effects of beta-tACS on corticospinal excitability: A meta-analysis. *Brain Stimulation*, 12(6), 1381-1389. <https://doi.org/https://doi.org/10.1016/j.brs.2019.07.023>
- Wong, J. D., Kistemaker, D. A., Chin, A., & Gribble, P. L. (2012). Can proprioceptive training improve motor learning? *J Neurophysiol*, 108(12), 3313-3321. <https://doi.org/10.1152/jn.00122.2012>
- Yamaguchi, T., Svane, C., Forman, C. R., Beck, M. M., Geertsen, S. S., Lundbye-Jensen, J., & Nielsen, J. B. (2020). Transcranial alternating current stimulation of the primary motor cortex after skill acquisition improves motor memory retention in humans: a double-blinded sham-controlled study. *Cerebral Cortex Communications*, 1(1), tgaa047.
- Yamamoto, S., Golanov, E. V., & Reis, D. J. (1993). Reductions in focal ischemic infarctions elicited from cerebellar fastigial nucleus do not result from elevations in cerebral blood flow. *Journal of Cerebral Blood Flow & Metabolism*, 13(6), 1020-1024.
- Yang, S., Yi, Y. G., & Chang, M. C. (2023). The effect of transcranial alternating current stimulation on functional recovery in patients with stroke: a narrative review. *Front Neurol*, 14, 1327383. <https://doi.org/10.3389/fneur.2023.1327383>
- Yarkoni, T., Poldrack, R. A., Nichols, T. E., Van Essen, D. C., & Wager, T. D. (2011). Large-scale automated synthesis of human functional neuroimaging data. *Nature methods*, 8(8), 665-670.
- Yu, M., Song, H., Huang, J., Song, Y., & Liu, J. (2020). Motor Learning Improves the Stability of Large-Scale Brain Connectivity Pattern [Original Research]. *Frontiers in Human Neuroscience*, 14. <https://doi.org/10.3389/fnhum.2020.571733>
- Yu, M., & Wang, S.-M. (2021). Anatomy, head and neck, occipital nerves. In *StatPearls [Internet]*. StatPearls Publishing.

- Yuan, K., Chen, C., Lou, W.-T., Khan, A., Ti, E. C.-H., Lau, C. C.-Y., Wang, X., Chu, W. C.-W., & Tong, R. K.-Y. (2022). Differential effects of 10 and 20 Hz brain stimulation in chronic stroke: a tACS-fMRI study. *IEEE transactions on neural systems and rehabilitation engineering*, 30, 455-464.
- Zapparoli, L., Mariano, M., & Paulesu, E. (2022). How the motor system copes with aging: a quantitative meta-analysis of the effect of aging on motor function control. *Communications Biology*, 5(1), 79. <https://doi.org/10.1038/s42003-022-03027-2>
- Zhang, X., Wang, H., Guo, Y., & Long, J. (2024). Beta rebound reduces subsequent movement preparation time by modulating of GABAA inhibition. *Cerebral Cortex*, 34(2). <https://doi.org/10.1093/cercor/bhae037>
- Zhu, F. F., Yeung, A. Y., Poolton, J. M., Lee, T. M. C., Leung, G. K. K., & Masters, R. S. W. (2015). Cathodal Transcranial Direct Current Stimulation Over Left Dorsolateral Prefrontal Cortex Area Promotes Implicit Motor Learning in a Golf Putting Task. *Brain Stimulation*, 8(4), 784-786. <https://doi.org/https://doi.org/10.1016/j.brs.2015.02.005>

9 Appendices

9.1 Appendix I: Supplemental information for Chapter 3

9.1.1 Evaluation of robustness

The results of the jackknife analysis showed that in each of the online and offline study groups, there was one cluster in which all studies included contributed activation. For the online group, a cluster with centre of mass at MNI coordinates [0, -20, 17] had contributions from all studies, corresponding to the midpoint between thalami. The offline group produced a cluster with centre of mass at MNI coordinates [-1, -23, 12] had contributions from all offline studies, likewise attributed to the midpoint between thalami. All cluster values are presented in Supplemental Tables 9.1 and 9.2. Thus, our results illustrate that both online and offline scanning studies included implicated activation changes in (bilateral) thalami.

Table 9.1 Online motor learning study cluster values, center of mass and contribution.

Cluster ID											
1	2	3	4	5	6	7	8	9	10	11	
Center of mass coordinates (MNI)											
	(0, -21, 17)	(-49, 15, -17)	(-46, -2, -18)	(-3, -50, -42)	(3, -2, 18)	(16, -32, 79)	(20, -50, 18)	(40, -25, -19)	(42, 14, 32)	(52, -22, -16)	(61, -8, 27)
1	0.026309	0	0.000083	0.000778	0	0	0.00441	0.000194	0.000114	0.000015	0.000038
2	0.037543	0.011433	0	0	0	0	0	0	0	0	0
3	0.019329	0	0	0	0	0	0	0	0	0	0
4	0.091495	0.977349	0.002359	0.986495	0.00258	0.004873	0	0.021709	0.000011	0.988451	0.988454
5	0.01244	0	0	0	0	0	0	0	0	0	0
6	0.128828	0	0	0	0.13523	0	0.000228	0.000289	0.000636	0	0
7	0.016699	0.000085	0	0	0	0	0.005594	0	0	0	0
8	0.017341	0	0	0	0	0	0.00141	0.000002	0	0.002647	0.01145
9	0.024298	0	0	0	0	0	0	0	0.000747	0	0
10	0.013702	0	0	0.000088	0	0.993178	0	0	0	0	0
11	0.013304	0	0.000006	0	0.000025	0	0	0.000006	0	0	0
12	0.03285	0	0.000001	0.005943	0.000006	0	0	0.977001	0.000002	0.008806	0
13	0.015837	0	0	0	0.000024	0	0.000001	0.000152	0.000004	0.000002	0
14	0.0647	0.009168	0.993125	0	0	0.000017	0.000529	0	0.998042	0	0
15	0.014101	0	0	0.000002	0	0.00189	0	0	0	0	0
16	0.018628	0	0	0.000405	0	0.000002	0	0	0	0	0
17	0.015631	0	0	0	0	0	0	0	0	0	0
18	0.029939	0	0	0.005516	0.000031	0	0	0.000227	0	0	0
19	0.016626	0	0.000014	0.000677	0	0	0	0	0	0	0
20	0.094213	0.000045	0.000324	0	0.861583	0	0.004631	0.000006	0.000185	0.000001	0
21	0.033719	0.000534	0	0	0	0	0	0	0	0	0

22	0.016874	0	0	0	0	0	0.001364	0.000275	0	0	0
23	0.005713	0	0	0.000032	0	0	0	0	0	0	0
24	0.01278	0	0.000041	0	0	0	0.000003	0	0	0	0
25	0.048638	0.000011	0.00394	0	0	0.000002	0.000638	0	0	0.000012	0.000001
26	0.039531	0.001199	0	0	0	0	0.980524	0	0	0	0
27	0.016976	0	0	0	0	0	0	0	0	0	0
28	0.003491	0	0	0	0	0	0	0	0	0	0
29	0.024103	0	0.000005	0	0	0	0.000001	0	0	0	0
30	0.04122	0	0.000022	0	0	0	0	0	0	0.00001	0
31	0.048362	0.000062	0.000046	0	0.000002	0	0.000571	0.00002	0.000247	0	0
32	0.001649	0	0	0	0	0	0	0	0	0	0

Table 9.2 Offline motor learning study cluster values, center of mass, and contribution.

	Cluster ID	
	1	2
	Center of mass coordinates (MNI)	
	(-1, -23, 12)	(32, -11, 66)
33	0.147237	0
34	0.128299	0
35	0.188739	0.000008
36	0.166548	0
37	0.264259	0.999992
38	0.104837	0

9.1.2 Availability of data, code, and materials

For database search and analysis, NiMARE (<https://github.com/neurostuff/NiMARE>) was used, integrating both Neurosynth (<https://github.com/neurosynth/neurosynth>) and Neuroquery (<https://github.com/neuroquery>) databases. Plotting was performed using the nilearn package (<https://github.com/nilearn>).

9.2 Appendix II: Supplemental information and data for Chapter 4

9.2.1 Supplemental figures

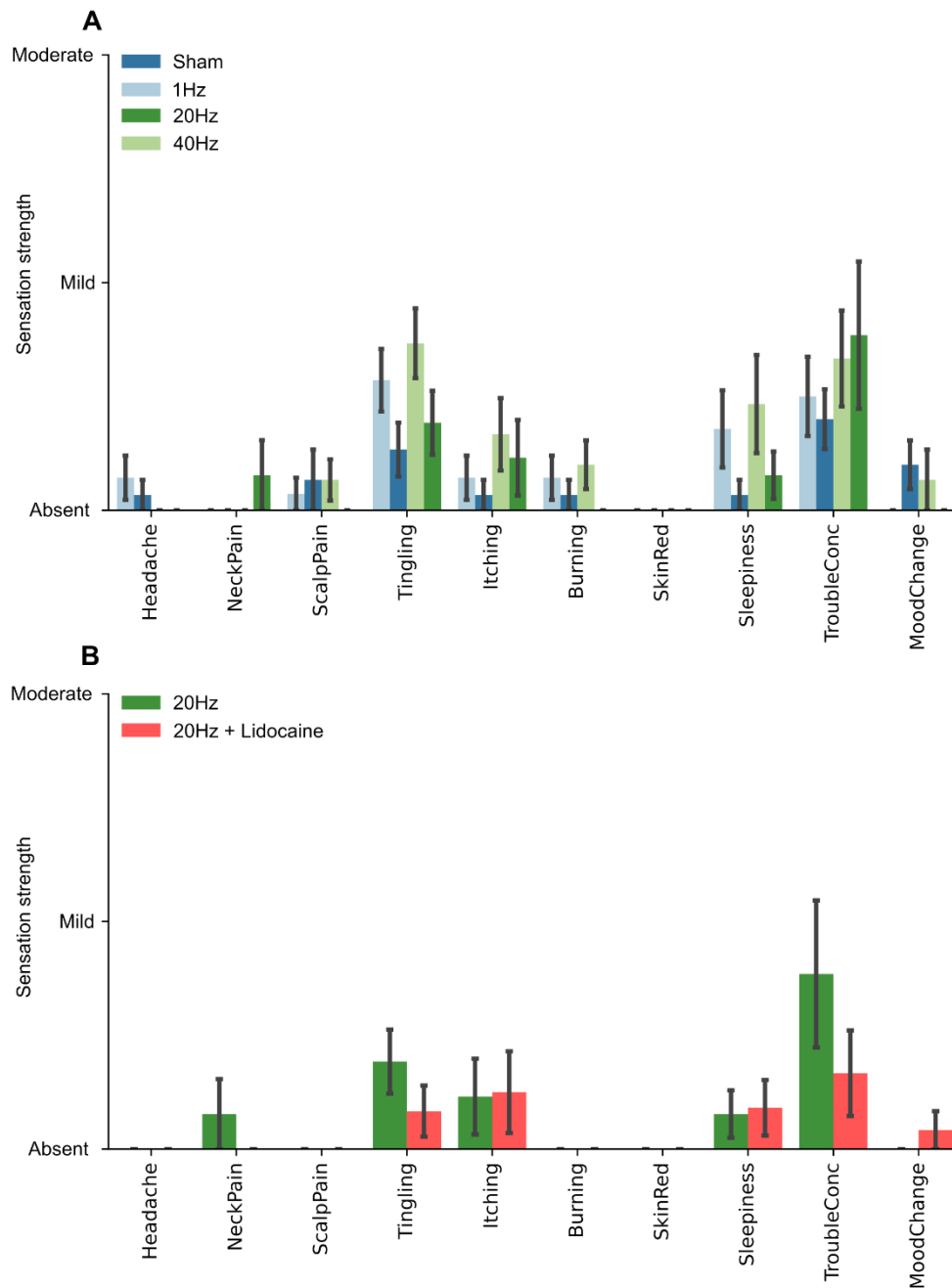


Figure 9.1 (A) Sensation reporting for experiment 1 groups following stimulation. (B) Sensation reporting for experiment 1 follow-up comparing 20 Hz and 20 Hz + Lidocaine group. Mean response is shown, error bars denote SEM.

9.2.2 Supplementary data

Table 9.3 Demographic, BDI, and BAI scores by group included in experiment 1 analyses.

Group	N	Mean age ± SD	Mean BDI ± SD	Mean BAI ± SD	Gender	Handedness^a
Sham	15	24.0 ± 6.5	3.3 ± 3.9	3.3 ± 3.0	12F/3M	4L/11R/0M
1 Hz	14	21.1 ± 3.8	2.4 ± 4.2	3.2 ± 4.6	13F/1M	1L/13R/0M
20 Hz	13	25.7 ± 3.2	6.4 ± 7.9	8.3 ± 10.1	8F/5M	0L/12R/1M
40 Hz	15	21.1 ± 3.0	7.2 ± 6.1	9.2 ± 7.1	11F/4M	2L/12R/1M

^aL = left, R = right, M= mixed.

Table 9.4 Demographic, BDI, and BAI scores by group included in additional analyses.

Group	N	Mean age ± SD	Mean BDI ± SD	Mean BAI ± SD	Gender	Handedness^a
20 Hz + Lidocaine	13	25.9 ± 3.1	5.5 ± 5.1	6.5 ± 5.6	8F/5M	0L/13R/0M

^aL = left, R = right, M= mixed.

9.2.3 Questionnaires

Supplementary Data 9.2.1 Edinburgh Handedness Inventory (Short Version)

Edinburgh Handedness Inventory – Short Form					
Please indicate your preference in the use of hands for use of the following:					
	Always R	Usually R	Both Equal	Usually L	Always L
Writing					
Throwing					
Toothbrush					
Spoon					

Below for researcher:

Writing Score:

Throwing Score:

Toothbrush Score:

Spoon Score:

Total :

L.Q. (Total/4) :

Classification:

Left hander: -100 to -61

Mixed hander: -60 to 60

Right hander: 60 to 100

Supplementary Data 9.2.2 tES exit-questionnaire

tDCS Exit Questionnaire

Please indicate which, if any, symptoms or side-effects you felt after tES. If present, please then indicate how strongly it related to tES by writing: 1 = none, 2 = remotely, 3 = possibly, 4 = probably, or 5 = definitely

	1 = Absent	2 = Mild	3 = Moderate	4 = Severe	IF PRESENT, Related to tES?
Headache	☐	☐	☐	☐	_____
Neck Pain	☐	☐	☐	☐	_____
Scalp Pain	☐	☐	☐	☐	_____
Tingling	☐	☐	☐	☐	_____
Itching	☐	☐	☐	☐	_____
Burning Sensation	☐	☐	☐	☐	_____
Skin Redness	☐	☐	☐	☐	_____
Sleepiness	☐	☐	☐	☐	_____
Trouble Concentrating	☐	☐	☐	☐	_____
Acute Mood Changes	☐	☐	☐	☐	_____
Other (Please Specify) _____	☐	☐	☐	☐	_____

(Suggested questionnaire by Brunoni et al., 2011)

In which group do you think you are in?

☐ Active

☐ Non-active

ADDITIONAL COMMENTS

Folder A

9.3 Appendix III: Supplemental data for Chapter 5

Table 9.4 Demographic, BDI, and BAI scores by group included in analyses.

Group	N	Mean age ± SD	Mean BDI ± SD	Mean BAI ± SD	Gender	Handedness
Sham	15	21.6 ± 2.1	5.5 ± 8.8	7.7 ± 10.5	12F/3M	3L/12R
20 Hz	15	21.5 ± 1.4	5.0 ± 5.1	4.2 ± 3.8	15F/0M	4L/11R

Table 9.5 Participant exclusion by data source.

Data	N (Sham)	N (20 Hz)
Behavioural (day 1)	15	15
Behavioural (day 2)	15	15
rsEEG	15	14
Day 1 active EEG	15	15
Day 2 active EEG (trained)	15	13
Day 2 active EEG (untrained)	15	12
Eyetracking	12	10

Table 9.6 Contralateral random trial analyses for PMBR

Contrast	Sham stimulation		20 Hz stimulation	
	Test statistic	p_{corr}	Test statistic	p_{corr}
Pre-train –Post-train	1.363 ^a	1.00	0.830	1.00
Pre-train –Trained Day 2	1.193 ^a	1.00	0.734 ^a	1.00
Pre-train –Untrained Day 2	1.136 ^a	1.00	0.235 ^a	1.00
Post-train –Trained Day 2	-0.17 ^a	1.00	0.454 ^a	1.00
Post-train –Untrained Day 2	-0.45 ^a	1.00	0.784 ^a	1.00
Trained Day 2 –Untrained Day 2	0.227 ^a	1.00	0.254	1.00

^a Wilcoxon rank-sum z .

Table 9.7 Ipsilateral random trial analyses for PMBR

Contrast	Sham stimulation		20 Hz stimulation	
	Test statistic	p_{corr}	Test statistic	p_{corr}
Pre-train –Post-train	0.475	1.00	1.812	0.54
Pre-train –Trained Day 2	0.114 ^a	1.00	0.463	1.00
Pre-train –Untrained Day 2	0.625 ^a	1.00	-0.628 ^a	1.00
Post-train –Trained Day 2	-0.795 ^a	1.00	-1.013 ^a	1.00
Post-train –Untrained Day 2	-0.170 ^a	1.00	-0.955	1.00
Trained Day 2 –Untrained Day 2	0.797	1.00	-0.105	1.00

^a Wilcoxon rank-sum z .

Table 9.8 Post-hoc analysis of ERDS for Group $\times$ Side comparisons. Shaded rows denote contrasts of interest. Significant contrasts are bolded.

Contrasts		Mean difference	SE	T	p_{corr}^a
20 Hz, Contra	Sham, Contra	5.524×10^{-17}	7.570×10^{-17}	0.73	1
	20 Hz, Ipsi	-113.22	2.880×10^{-17}	-3.341	0.016
	Sham, Ipsi	4.328×10^{-17}	7.570×10^{-17}	0.572	1
Sham, Contra	20 Hz, Ipsi	-31.15	7.570×10^{-17}	-2.001	0.332
	Sham, Ipsi	-28.96	2.667×10^{-17}	-0.449	1
20 Hz, Ipsi	Sham, Ipsi	1.395×10^{-16}	7.570×10^{-17}	1.843	0.457

^a p -values are adjusted for multiple comparisons (Bonferroni correction family of 6).

9.4 Appendix IV: Supplemental information for Chapter 6

Supplementary Data 9.4.1 Edinburgh Handedness Inventory (Short Version) (Dutch Translation)

PARTICIPANT ID: _____

DATE: _____

Edinburgh Handedness Inventory - korte vorm

Geef uw voorkeur aan voor het gebruik van handen voor het gebruik van de volgende zaken:

	Altijd R	Meestal R	Beide handen	Meestal L	Altijd L
Schrijven					
Werpen					
Tandenborstel					
Lepel					

Below for researcher:

Writing Score:

Throwing Score:

Toothbrush Score:

Spoon Score:

Total :

L.Q. (Total/4) :

Classification:

Left hander: -100 to -61

Mixed hander: -60 to 60

Right hander: 60 to 100

Researcher Initials: _____

Table 9.9. Chapter 6 demographics by stimulation condition.

	1 Hz, n = 7	20 Hz, n = 7	<i>P</i>_{corr}
	Mean (SD)	Mean (SD)	
Age (years)	41.143 (15.774)	39.714 (3.115)	1.00 ^a
Sex (male/female)	4/3	3/4	1.00 ^b
BDI	6.286 (5.992)	6.857 (6.040)	1.00 ^a
BAI	5.857 (4.981)	5.143 (4.180)	1.00 ^a
Handedness (left/right/mixed)	2/5/0	0/7/0	0.64 ^b

^aOne-way ANOVA^bChi-square test