Effects of Trigeminal Nerve Stimulation on Visuomotor Learning

by

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ABSTRACT

A current thrust in neurorehabilitation research involves exogenous neuromodulation of peripheral nerves to enhance neuroplasticity and maximize recovery of function. This dissertation presents the results of four experiments aimed at assessing the effects of Trigeminal Nerve Stimulation (TNS) and Occipital Nerve Stimulation (ONS) on motor learning, which was behaviorally characterized using an upper extremity visuomotor adaptation paradigm.

In Aim 1A, the effects of offline TNS using clinically tested frequencies (120 and 60 Hz) were characterized. Sixty-three participants (22.75±4.6 y/o), performed a visuomotor rotation task and received TNS before encountering rotation of hand visual feedback. In Aim 1B, TNS at 3 kHz, which has been shown to be more tolerable at higher current intensities, was evaluated in 42 additional subjects (23.4±4.6 y/o). Results indicated that 3 kHz stimulation accelerated learning while 60 Hz stimulation slowed learning, suggesting a frequency-dependent effect on learning.

In Aim 2, the effect of online TNS using 120 and 60 Hz were characterized to determine if this protocol would deliver better outcomes. Sixty-three participants (23.2±3.9 y/o) received either TNS or sham concurrently with perturbed visual feedback. Results showed no significant differences among groups. However, a cross-study comparison of results obtained with 60 Hz offline TNS showed a statistically significant improvement in learning rates with online stimulation relative to offline, suggesting a timing-dependent effect on learning.

In Aim 3, TNS and ONS were compared using the best protocol from previous aims (offline 3 kHz). Additionally, concurrent stimulation of both nerves was explored to look for potential synergistic effects. Eighty-four participants (22.9±3.2 y/o) were assigned to one of four groups: TNS, ONS, TNS+ONS, and sham. Visual inspection of learning curves revealed that the ONS group demonstrated the fastest learning

among groups. However, statistical analyses did not confirm this observation. In addition, the TNS+ONS group appeared to learn faster than the sham and TNS groups but slower than the ONS only group, suggesting no synergistic effects using this protocol, as initially hypothesized.

The results provide new information on the potential use of TNS and ONS in neurorehabilitation and performance enhancement in the motor domain.

DEDICATION

To Astrid and Catalina, whose unconditional love and support have been the source of my strength throughout this journey. And to my parents, for their unending love and unwavering support throughout my life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

ABBREVIATIONS

ACC Anterior Cingulate Cortex.

ADHD Attention-Deficit/Hyperactivity Disorder.

DE Directional Error.

DRE Drug-Resistant Epilepsy.

FDA Food and Drug Administration.

FMA-UE Upper Extremity Fugl-Meyer Assessment.

IAPS International Affective Picture System.

KES Kilohertz Electrical Stimulation.

LC Locus Coeruleus.

NE Norepinephrine.

NTS Nucleus Tractus Solitarius.

ONS Occipital Nerve Stimulation.

PFC Prefrontal Cortex.

PV Peak Velocity.

RT Reaction Time.

SI Somatosensory Cortex.

tACS Transcranial Alternating Current Stimulation.

taVNS Transcutaneous Auricular Vagus Nerve Stimulation.

TCC Trigeminocervical Complex.

tcVNS Transcutaneous Cervical Vagus Nerve Stimulation.

tDCS Transcranial Direct Current Stimulation.

TENS Transcutaneous Electrical Nerve Stimulation.

TMS Transcranial Magnetic Stimulation.

TNS Trigeminal Nerve Stimulation.

VNS Vagus Nerve Stimulation.

VPM Ventral Posteromedial Nucleus of the Thalamus.

Chapter 1

INTRODUCTION

1.1 Motivation

A large body of evidence supports the use of neuromodulation as a potential treatment for neurological disorders. Neuromodulation has also shown promise as a means to enhance cognitive and other functions, including learning. Although initial work focused on directly stimulating the brain (e.g., TMS, tDCS), recent studies have shown that neuroplasticity and learning can also be enhanced via the stimulation of cranial nerves. For example, Vagus Nerve Stimulation (VNS) has shown the ability to enhance plasticity in motor areas [1], leading to exploring its potential for stroke rehabilitation. Recent studies in stroke patients with upper limb dysfunction have found that those receiving VNS in addition to conventional therapy showed greater functional improvement than patients receiving sham VNS and therapy [2]. Despite these reported beneficial effects, patients might still be reluctant to use VNS because it requires a surgically implanted electrode. Additionally, there are concerns regarding cost, accessibility, and side effects [3, 4]. Thus, more research needs to be done on VNS as well as other non-invasive methods that could potentially exert similar effects.

TNS is a novel neuromodulation tool that emerges from the need for non-invasive alternatives to VNS. The supraorbital branches of the trigeminal nerve are distributed superficially, making them easily accessible using surface electrodes. The trigeminal nerve projects to many of the same anatomical structures as the vagus nerve, i.e., both connect with brainstem nuclei such as the Nucleus Tractus Solitarius (NTS) and Locus Coeruleus (LC), which influence cortical networks involved in arousal and cognition.

This activation pathway is the main hypothesis regarding the mechanism for VNS and TNS effects. Although TNS has a similar therapeutic profile as VNS, with beneficial effects on Drug-Resistant Epilepsy (DRE), Attention-Deficit/Hyperactivity Disorder (ADHD), migraine, and others, TNS's potential to enhance learning, particularly in the motor domain, remains unknown.

In addition to TNS, ONS is another potential candidate to activate brainstem structures that can modulate activity in cortical areas. The occipital nerves arise from the cervical spinal afferents at the level of C2/C3 that converge with trigeminal nerve afferents at the trigeminal cervical complex, which projects to the LC and NTS. ONS has been used in clinical settings for migraine, and it is currently under investigation for memory improvement in older adults [5]. Recently, a novel approach combined trigeminal and occipital nerve stimulation to take advantage of the convergence of both nerves and potentially find a synergistic effect in migraine [6]. Thus, ONS may also have the potential to enhance cognitive functions such as learning and memory, motor learning but its effects on tasks in the motor domain have not been explored.

The studies described here will explore the ability of TNS and ONS to enhance motor learning in healthy adults. In the proposed experiments, we will test different frequencies of electrical stimulation, including those that have shown beneficial effects in ADHD, DRE, and migraine studies, as well as frequencies in the kilohertz range. Additionally, we will explore different timings of the TNS application (before vs during task performance) to determine which maximizes its effects. Finally, we will directly compare TNS and ONS effects on motor learning and explore potential synergistic effects of combining both modalities.

1.2 Specific Aims

Aim 1: Characterize the effects of offline (priming) electrical stimulation of the trigeminal nerve on visuomotor adaptation in healthy subjects.

In this aim, we will investigate the effects of a single session of TNS delivered transcutaneously prior to task performance (offline). To this end, healthy subjects will receive 10 minutes of effective TNS before engaging in a visuomotor adaptation task that requires adapting to a perturbation of their hand visual feedback. TNS will be delivered non-invasively by placing two electrodes on the forehead, targeting the supraorbital branches of the trigeminal nerve. In **Aim 1A**, we will test two frequencies that have been used clinically to ameliorate the symptoms of DRE, ADHD (120 Hz), and migraine (60 Hz). In **Aim 1B**, we will test an experimental protocol that explores frequencies in the kilohertz range (3 kHz) which is thought to minimize the activation of motor and pain fibers [7]. We hypothesize that offline stimulation will enhance attention and learning mechanisms resulting in better performance and faster learning than sham.

Aim 2: Characterize the effects of online electrical stimulation of the trigeminal nerve on visuomotor adaptation in healthy subjects.

In this aim, we will investigate the effects of a single session of TNS delivered transcutaneously during task performance (online). Unlike **Aim 1**, where the stimulation is delivered offline, TNS will be applied while subjects experience a perturbation of their visual feedback during a motor adaptation task. The investigation of online effects is important because it will allow us to determine which stimulation timing provides the best outcomes (online v/s offline). In this aim, we will use the same frequencies and task as in **Aim 1A**. We hypothesize that the effects of online stimulation on learning and attention will be more pronounced than the effects induced

by offline TNS.

Aim 3: Distinguish the effects of transcutaneous electrical stimulation of the trigeminal nerve, occipital nerve and both nerves on visuomotor adaptation.

In this aim, we will compare the TNS protocol that shows the best performance in Aims 1 and 2 with the stimulation of the occipital nerve. The trigeminal and the occipital nerves both converge onto the trigeminal sensory nuclear complex, thus either or both can potentially activate brainstem circuits responsible for endogenous modulation of the cerebral cortex [7]. These two nerves will be stimulated separately as well as simultaneously to see whether driving these pathways at the same time provides a better outcome on learning. To this end, three stimulation protocols (TNS, ONS, and TNS + ONS) will be tested, using the same motor adaptation task described in **Aim 1** and **2**. We hypothesize that TNS + ONS will show more pronounced effects on learning and performance than TNS and ONS individually, but all techniques will show stronger effects than sham.

1.3 Dissertation Overview

The present dissertation is structured into seven chapters. Chapter 1 begins by providing the motivation behind this work and outlining the specific aims.

Chapter 2 presents a comprehensive literature review, focusing on cranial-based stimulation techniques such as TNS, VNS, and ONS. This chapter explores their potential mechanisms of action and implications for motor learning.

Chapter 3 provides an overview of the methods employed throughout the experiments. It details the behavioral task selected, participants' selection criteria, system overview, data collection procedures, and data analysis.

Chapter 4 describes two studies conducted to address Aim 1, which investigates the use of offline TNS on visuomotor learning. Aim 1A examines stimulation frequencies at 120 Hz and 60 Hz, which have shown therapeutic effects in clinical trials for ADHD and migraine. Aim 1B explores an experimental protocol using frequencies in the kilohertz range on a distinct cohort of participants.

Chapter 5 addresses Aim 2, where the same frequencies used in Aim 1B (120 and 60 Hz) were delivered online. Results from both studies were compared (Aim 1A and 2) to determine whether offline or online stimulation produced the best outcomes on motor learning.

Chapter 6 presents the results of the study conducted to address Aim 3. In this experiment, the best TNS protocol from previous aims was compared with ONS. Additionally, it was investigated whether concurrent stimulation of both nerves (TNS+ONS) would have stronger effects on learning compared to independent stimulation (TNS or ONS).

Lastly, Chapter 7 concludes the dissertation with a general discussion that summarizes key findings, presents conclusions drawn from the studies, and suggests potential directions for future research in the field of cranial-based non-invasive stimulation.

Chapter 2

BACKGROUND

2.1 Introduction

Pharmacological and non-pharmacological techniques have been used to modulate activity in the brain. Among the non-pharmacological methods, one approach is to stimulate the cortex directly to evoke a cortical response. Transcranial Direct Current Stimulation (tDCS) and Transcranial Magnetic Stimulation (TMS) are examples of these techniques. These methods try to depolarize brain areas in a top-down fashion using electric current and magnetic fields, respectively. Another approach is to activate the cortex indirectly via the stimulation of bottom-up pathways. Several studies have shown that it is possible to generate brain excitability by applying electrical stimulation to specific peripheral nerves such as the vagus (Cranial Nerve X), trigeminal (Cranial Nerve V), and occipital nerves. As shown in Fig.2.1, sensory projections from these nerves ascend to a central hub in the brain stem hub linked to the Nucleus Tractus Solitarius (NTS), which project to several nuclei responsible for neurotransmitter release, i.e., the Locus Coeruleus (LC), which is the main source of Norepinephrine (NE), a neurotransmitter that play a significant role in cortical plasticity and behaviour related to memory and learning.

Vagus Nerve Stimulation (VNS) is currently approved for stroke rehabilitation, setting a precedent for this bottom-up mechanism to modulate motor behavior. However, it is unknown whether the trigeminal and occipital nerves, which share anatomical projections, would produce similar results. Thus, this dissertation will gather important information to assess the feasibility of their use in the motor domain.

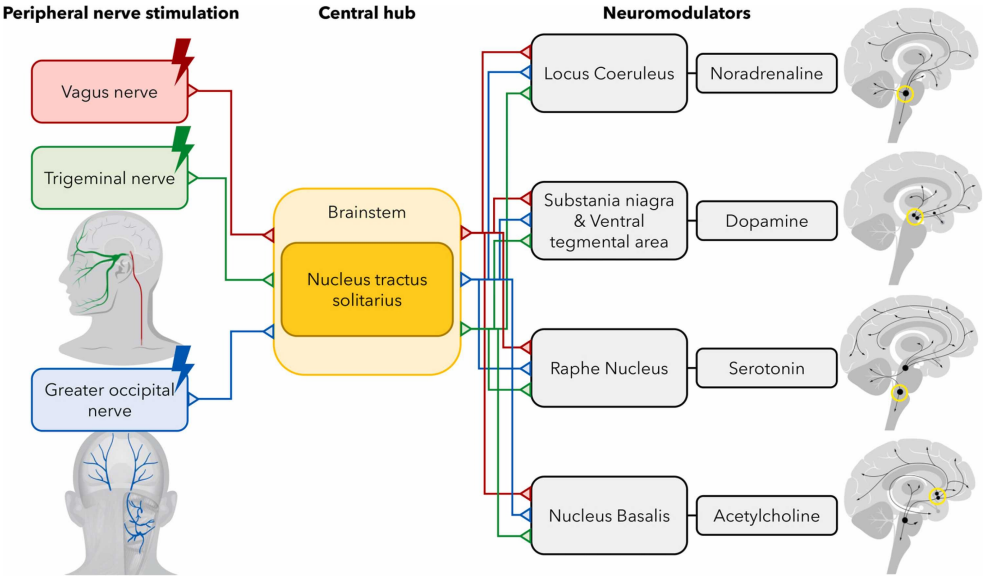

Figure 2.1: Vagus, trigeminal and occipital nerve stimulation activate NTS, which projects to multiple neurotransmitters centers (Figure taken from [8]).

In this chapter, we will provide an overview of VNS in the context of the motor domain. Next, we will explore the feasibility of using non-invasive Trigeminal Nerve Stimulation (TNS) and Occipital Nerve Stimulation (ONS) to enhance motor behavior. Finally, we will delve into the importance of motor learning and its link with plasticity.

2.2 Vagus Nerve Stimulation

VNS stands out as a prominent technique in cranial nerves-based methods to neuromodulate the brain. The electrical stimulation of the vagus nerve with low-intensity currents has gained recognition as an effective neuromodulatory approach to ameliorate the symptoms of Drug-Resistant Epilepsy (DRE) and difficult-to-treat depression, among others. This technique required the implantation of a cuff electrode in the left cervical vagus connected to a generator implanted subcutaneously in the chest. VNS received Food and Drug Administration (FDA) approval for DRE in

1997 and for difficult-to-treat depression in 2005. In 2021, VNS was approved for the rehabilitation of ischemic stroke with upper limb dysfunction, thanks to many years of research that shows that VNS can modulate neuroplasticity and motor behavior.

The interest in VNS for stroke rehabilitation emerged from studies that demonstrated in animal models that pairing VNS with a specific movement increases the representation of that movement in the primary motor cortex, suggesting that VNS can promote neuroplasticity [1]. Subsequently, a series of studies investigated the feasibility of using VNS to enhance rehabilitation. For instance, in [9], to model the effect of an ischemic stroke, mice were subjected to subcortical ischemic lesions to impair the performance of a reaching task previously trained for several weeks. Subsequently, a subgroup, randomly chosen, was implanted with a VNS device. Following a one-week recovery period, the VNS and control (injured but not device implanted) groups underwent 6 weeks of rehabilitation. Results revealed that the group under VNS treatment showed better performance in the reaching task post-rehabilitation.

Based on the promising results of VNS in animal models, the first pilot studies in patients began. For example, in [10], 20 ischemic stroke survivors with upper limb impairment were randomly assigned into two groups, one group was selected to be implanted with a VNS device ($n = 9$) and, the other group was used as a control ($n = 11$). After implantation, all participants underwent 6 weeks of rehabilitation consisting of 3 sessions per week, 2 hours long in which subjects perform task-specific movements. Particularly, the implanted group received VNS paired with movement onset and triggered manually by a physical therapist. Patients' improvements were assessed at baseline and post-rehabilitation using the Upper Extremity Fugl-Meyer Assessment (FMA-UE). The results showed that the VNS group increased on average, 9.6 points in the FMA-UE compared with baseline, which is considered clinically meaningful. These findings were further supported by randomized, double-blinded,

sham-controlled study [11] involving stroke patients who received either real (n = 11) or sham (n = 9) VNS followed by 6 weeks of in-clinic therapy and subsequent home-based therapy for three months. FMA-UE scores assessed at 1, 30, and 90 days after VNS implantation revealed an increase of 7.6 points for real VNS and 5.3 points for sham on day 1 post–in-clinic therapy. After 90 days, average scores increased by 9.5 points for real VNS, while sham scores improved by 3.8 points. These series of studies were explored further to achieve a statistical power of 80%. Thus, a multi-site, double-blinded randomized sham-controlled study that included 108 stroke patients found that those receiving VNS in addition to conventional therapy showed greater functional improvement than patients receiving sham VNS and therapy [2]. Consequently, these significant findings, along with related research, led the FDA to approve VNS for the treatment of upper limb dysfunction associated with chronic and ischemic stroke.

Although the application of VNS for stroke is among the most promising recent developments in the rehabilitation of stroke, might not be widely adopted because it requires a surgical procedure which might generate concerns regarding cost, accessibility, and side effects, as well as other factors [3, 4]. Thus, VNS might not be suitable for all patients. For this reason, several research groups have explored non-invasive VNS alternatives in different areas. For instance, a hand-held Transcutaneous Cervical Vagus Nerve Stimulation (tcVNS) device was recently approved by the FDA for migraine and cluster headaches (gammaCore, electroCore LLC., Basking Ridge, New Jersey) and it is also under investigation for stroke patients [12]. Additionally, there is an active research area studying the auricular branches of the vagus nerve, which has shown encouraging results. For instance, in [13], 13 ischemic stroke survivors with upper limb dysfunction stroke showed an increase of 17.1$\pm$7.8 points in the FMA-UE scores after 18 rehabilitation sessions consisting of one hour

of repetitive task-specific movements paired with Transcutaneous Auricular Vagus Nerve Stimulation (taVNS). In [14], a similar cohort of stroke patients show improvements in FMA-UE score compared to sham (taVNS: 5.4 v/s sham: 2.8 points, $p = 0$ 048) after 2 weeks of rehabilitation where taVNS was combined with robotic-assisted therapy. This result suggests that taVNS may improve rehabilitation outcomes in stroke patients.

Although non-invasive VNS alternatives may be able to circumvent some of these issues, several lines of evidence indicate that the stimulation of other cranial nerves such as the trigeminal and the occipital nerve, might also produce similar neuromodulatory effects. VNS is thought to exert its beneficial effects on neuroplasticity through activation of brainstem structures responsible for arousal and endogenous neuromodulation of the cerebral cortex, such as the LC [15, 16]. The LC in turn sends mostly excitatory inputs to cortical areas involved in arousal, attention, and stress responses as well as cognitive and other functions such as learning [17]. However, the vagus nerve is not the only cranial nerve capable of accessing these cortical networks. Like the vagus, the trigeminal and occipital nerves project to the LC via the NTS and also through the sensory trigeminal nerve nuclei [18, 19].

Therefore, the trigeminal and the occipital nerves provided two viable alternatives for enhancing learning in the motor domain, given their similar anatomical projections and potentially the same mechanism of action.

2.3 Trigeminal Nerve Stimulation

TNS is a novel neuromodulation technique that rise as alternative to VNS where low-intensity electrical stimulation is delivered to the branches of the trigeminal nerve to indirectly modulate brain activity. The trigeminal nerve is the fifth cranial nerve and carries sensory information about touch, pain, temperature, and proprioception

from the face to the brain but also has motor fibers that innervate the muscles of mastication. It is divided into three branches: Ophthalmic (V1), Maxillary (V2), and Mandibular (V3). V1 and V2 only contain sensory afferents, and V3 contains both motor efferents and sensory afferents. The sensory afferents from the three branches converge into the trigeminal ganglion (also known as Gasserian Ganglion or Semilunar Ganglion). From there, their axons are sent to the brain stem to synapse with the trigeminal sensory nuclear complex. This region is formed by the mesencephalic nucleus, the principal sensory nucleus (also called pontine, main sensory or chief sensory nucleus) and the spinal nucleus. From the trigeminal sensory nuclear complex, projections are sent to the Ventral Posteromedial Nucleus of the Thalamus (VPM) via the trigemino-thalamic tract and from there to Somatosensory Cortex (SI).

In addition, studies in animal models indicate that afferents of the trigeminal nerve also project to different brain stem nuclei such as the NTS, LC, the dorsal raphe nucleus and the pedunculopontine nucleus [20, 21]. These structures project to different areas of the cortex and modulate their function by the release of neurotransmitters. For instance, LC is the major source of NE, the the dorsal raphe nucleus is the primary source of serotonin, and the pedunculopontine nucleus releases acetylcholine. These brain structures are part of the reticular activating system known for its functions in attention, arousal, learning, awareness among other functions. Although the mechanism of action is not well understood, the main hypothesis explaining TNS effects is the activation of ascending trigeminal pathways described above. Additionally, simulation studies have shown that TNS effects could be associated with a frontal cortex activation [22].

Therapeutic effects observed in clinical trials support the hypothesis of a bottom-up mechanism that alters the cortex. For instance, the interest of TNS in Attention-Deficit/Hyperactivity Disorder (ADHD) patients comes from observations made in

clinical studies related to mood disorders [23], where subjects showed improvement in attention and concentration after TNS. Pharmacological treatments for ADHD facilitate dopamine and NE transmission to the prefrontal cortex, which has been shown to improve concentration and attention [24]. Thus, the beneficial effects observed in ADHD after TNS could be explained by facilitating NE release. Evidence from imaging studies supports the ability to produce plastic changes in the brain. For instance, PET imaging studies have shown that TNS produces activation in the Anterior Cingulate Cortex (ACC), frontal cortex, parieto-temporal cortex, all brain regions implicated in ADHD [23]. Moreover, in [25], migraine patients show similar activation patterns after three months of TNS. Hypometabolism in the orbital frontal cortex and the ACC was found at baseline, which showed close to normal activity after three months of TNS. Changes in cortical activation correlated with clinical improvements.

TNS has been shown to improve the symptoms of ADHD [23], major depression disorder (MDD) [26], DRE [27], migraine [28], and other disorders. These studies had led to the approval of TNS devices to the market. For example, the Monarch eTNS system (Neurosigma Inc., Los Angeles, CA) for pediatric ADHD have obtained FDA approval for patients between 7-12 years old who are not currently taking any ADHD prescriptions [29]. It is also approved for DRE in adults and children 9 years old and older in Europe [30] and Canada [31]. In the U.S, the FDA has granted it Breakthrough Device Designation for DRE allowing patients to have a quicker access to this treatment [32]. Another TNS device known as Cefaly (Seraing, Belgium) is approved in the U.S, Canada, and Europe for treating and prevention of migraines [33]. Thus, although TNS is currently approved for the treatment of several neurological disorders and neuroanatomical data suggest that TNS could have potent neuromodulatory effects on cortical networks involved in cognition, very few studies have directly

assessed the effects of TNS on learning and related cognitive functions, which would provide critical information regarding its potential use in neurorehabilitation.

2.3.1 Effects of TNS in Physiology

Studies conducted in animal models of epilepsy have provided evidence that TNS nerve can reduce pentylenetetrazole (PTZ) induced seizures [18, 21]. To this end, a nerve cuff was implanted either unilaterally or bilaterally in the infraorbital. Field potentials recording from the SI and the VPM showed that frequencies greater than 100 Hz generate a reduction in seizure activity by up to 78%. Also, thalamic and SI desynchronization was observed in the TNS group. Using a similar approach, in [21], they also observed seizure reduction. In addition, they provided insights into the brain circuitry affected by TNS using c-Fos immunostaining, an immunohistochemical technique to quantify neural activity. The results revealed significant activation at the trigeminal sensory nuclei and SI in the TNS group. It also showed activation at NTS, LC, dorsal raphe nucleus, amygdala, endopiriform nucleus, hippocampus, and entorhinal cortex, providing evidence that support the bottom-mechanism for TNS therapeutics effects.

Studies in humans have provided some insights regarding the effects of TNS in the brain. For instance, in [34], a paired-pulsed TMS protocol that stimulates the hand motor area was used to measure cortical excitability before, during, and after TNS. To this end, motor-evoked potentials were recorded from the first dorsal interosseus muscle while the hand motor area was stimulated using TMS. Results showed that TNS does not produce cortical excitability in the hand motor area. Thus, to obtain a broad picture of cortical changes induced by TNS, in [35], EEG was used to capture cortical changes while healthy subjects received a single session of TNS. To this end, EEG was measured before, during, and after a single session of bilateral TNS of

the infraorbital branches. Analysis of the spectral power and coherence of the EEG rhythms revealed intra-and interhemispheric coherence reduction in the beta band (13-30 Hz) during TNS. In addition, a similar trend in the gamma band (30-100 Hz) was also observed.These results indicate that TNS induces desynchronization of fast EEG waves with no topographic specificity which is in line with observations in animal models where cortical and thalamic desynchronization was observed [18, 21]. In [36], a PET imaging study assessed brain changes before and after TNS for 20 min in DRE patients. Results showed a statistically significant increase in cortical perfusion for the limbic and temporal areas, both areas highly involved in epilepsy.

2.3.2 Effects of TNS on Healthy Subjects

Healthy subjects exposed to acute TNS have reported sedative effects. In [37], subjects who received TNS using Cefaly for 20 minutes at 120 Hz showed a statistically significant decrease in performance on vigilance and attention tests compared with control, sham and subjects stimulated at 2.5 Hz for the same time. In [38], adverse events such as sleepiness/fatigue and sometimes insomnia were reported by 19 participants out of 2313 after 58 days using Cefaly with the 60 Hz/20 min protocol. In addition, [39] and [40] reported participants feeling sleepy and relaxed during and after TNS using the same stimulation protocol used by [37]. Based on these studies, [41] explored the sedative effects of TNS using the Multiple Sleep Latency Test. Although all the subjects reported drowsiness, no statistically significant differences were found between the experimental and sham groups. Therefore, it is unclear if TNS might produce sedative effects.

2.4 Occipital Nerve Stimulation

ONS is a neuromodulatory technique that stimulates the greater and lesser occipital nerves using small currents. Like VNS, ONS required the implantation of electrodes at the base of the neck connected to a power source implanted transcutaneously. Although it is not a therapy approved by the FDA, ONS has been used as an off-label label indication for pain prevention associated with different types of headaches [42]. Similar to VNS, the invasiveness of the procedure makes ONS not suitable for all patients, and non-invasive alternatives require further research.

Most applications targeting the occipital nerves are focused on migraine and headaches. For instance, in [43], transcutaneous ONS was used in patients with chronic migraine for three months, resulting in a significant decrease in the reduction of headaches and migraine days. Additionally, in [44], it was found that the response to Transcutaneous Electrical Nerve Stimulation (TENS) over the occipital nerve could serve as a predictor for therapeutic outcomes following ONS implantation. In a pilot study conducted by the same group, TENS over the occipital nerve was assessed in patients who experience refractory headaches after an intracranial vascular procedure. Results showed a reduction in pain levels after 6 months [45]. Thus, non-invasive alternatives targeting the occipital nerves show promise for headache and migraine patients.

Recent studies have shown that tDCS over the occipital nerve has the potential to activate brain circuits responsible for endogenous neuromodulation affecting memory circuits via the LC, similar to VNS and TNS [46]. Supporting this hypothesis, a recent study reported that tDCS applied over the occipital nerve enhanced associative memory in older adults [5]. In a double-blind sham-controlled study, the active group showed enhanced memory after 1 session and results persisted after 28 days. Also,

changes in α-amylase, which has been described as a NE biomarker, suggested the involvement of the LC-NE system. Therefore, transcutaneous stimulation of the occipital nerve might also provide a viable alternative to modulate cognitive and motor behavior.

2.5 Combined Neuromodulation Modalities

Novel approaches have attempted to boost neuromodulatory response by combining two different modalities to achieve strong behavioral or therapeutic effects. For instance, combined tDCS and taVNS have shown a tendency for pronounced improvements greater than the numerical summation of both modalities individually in a working memory task, suggesting a potential synergistic effect [47]. On the other hand, invasive ONS and TNS have been used for chronic patients unresponsive to current treatments with good results [48, 49]. A non-invasive alternative of this approach was recently approved by the FDA, combining non-invasive TNS and ONS in one device for the treatment of acute migraine [6]. Also, in [50], the supraorbital branches of the trigeminal nerve and the C2/C3 spinal afferents were stimulated to modulate stress response in human subjects by placing surface electrodes in the forehead and the back of the neck, following this approach of combined modalities.

The idea of combining modalities is particularly interesting for the trigeminal and occipital nerves. Anatomical and functional evidence from studies related to headaches and migraine mechanisms have suggested that both nerves converge into the Trigeminocervical Complex (TCC) which serves as a relay station from sensory information from the face, and head, including intracranial structures towards sensory processing structures [51]. Additionally, TCC sends projections to the NTS and LC, among other nuclei responsible for the modulation of different processes across the brain. Furthermore, these nerves have been proposed as responsible for part of the

effects observed in tDCS studies. The new theory suggests that part of the current injected by tDCS would be transmitted by the trigeminal and occipital nerves to brainstems structures such as NTS and LC, which would modulate cortical function [52]. Therefore, the stimulation of these nerves could offer a viable approach to alter LC activity and boost neuroplasticity in the context of learning, memory, and motor function. Hence, it merits further investigation.

2.6 Stimulation Parameters

2.6.1 Clinically Tested Frequencies

The most therapeutic effects for VNS, TNS, and ONS have been observed using frequencies ranging between 20-500 Hz. In particular for TNS, most studies have used three frequencies: 60, 100, and 120 Hz. The TNS at 120 Hz is the most commonly used today in clinical settings for DRE and ADHD. This parameter came from a study in animal models of epilepsy. In [18], several stimulation frequencies were tested to assess TNS anticonvulsant capabilities. They found that frequencies greater than 100 Hz reduce seizure activity by up to 78%. Additionally, TNS at 120 Hz is delivered in cycles of 30 seconds on and off seconds, which has been extensively used in invasive VNS. The main reason behind cyclic stimulation is to mitigate potential long-term damage associated with continuous stimulation, despite its potential for pronounced effects. Moreover, VNS side effects have been observed during the stimulation. Therefore, reducing the stimulation dose through ON/OFF cycles will reduce the probability of adverse events. Despite the rationale for cyclic stimulation applies more to an invasive stimulation procedure, these parameters have translated to the use of non-invasive TNS in clinical applications.

TNS and ONS delivered at 60 Hz have been used for migraines. For instance, the

device Cefaly has adopted the use of 60 Hz delivered continuously for 20 minutes to prevent migraines [28]. Another protocol from this device delivers continuous TNS at 100 Hz for 60 minutes to ameliorate the symptoms of chronic and acute migraine attacks. The therapeutic effects observed using 120 and 60 Hz TNS provide a good starting point to assess the feasibility of expanding its use for other applications.

2.6.2 Experimental Protocols: Kilohertz Electrical Stimulation

Kilohertz Electrical Stimulation (KES) is a novel approach, mostly used in spinal cord stimulation. For example, people with severe back pain have shown pain relief after spinal cord stimulation using up to 10 kHz [53]. Also, KES is an effective method to block nerve conduction, which has translated this technique into a non-pharmaceutical alternative for chronic pain. For instance, lower limb amputees suffering severe residual limb pain, or phantom limb pain have shown pain relief after receiving 5-10 kHz stimulation for 30 minutes over the sciatic or tibial nerve [54]. In addition, blocking the intraabdominal vagus nerve using KES have shown to reduce the appetite, leading to alternative therapy for people with obesity [55]. In all these cases, the stimulation was delivered directly through the targeted nerve, and the electrodes and the generator were surgically implanted.

On the other hand, non-invasive neuromodulatory approaches that have attempted to modulate behavior have also explored frequencies in the kilohertz range. For instance, Transcranial Alternating Current Stimulation (tACS) delivered between 1-5 kHz have been shown to increase excitability in the motor cortex [56]. Additionally, transcutaneous stimulation of the trigeminal and cervical spinal afferents delivered at 11 kHz showed to reduce sympathetic activity and physiological stress [7]. The same stimulation protocol showed to improve sleep quality and mood [50]. Furthermore, TNS delivered at 1 kHz was used in a study that examined the effects of both tcVNS

and TNS on learning, attention, and arousal in healthy adult subjects [57]. After a single session of stimulation, reaction times associated with the performance of a visual change detection task were found to be significantly faster with both tcVNS and TNS compared to sham, with effects that persisted even after 90 days. In [7, 50], the observed effects were associated with activation of the LC-NE system.

Additionally, KES protocols have shown to tolerate high intensities better relative to other non-invasive modulation techniques. For instance, in [7] currents ranging between 5-7 mA were applied without adverse events. This observation was associated with KES potentially minimizing the activation of motor and pain fibers. Thus, KES provide a valuable parameter to consider.

2.7 Motor Learning: Adaptation

To assess the effectiveness of TNS and ONS in the motor domain, we focused our attention on visuomotor adaptation, a type of learning where motor commands are altered to compensate for disturbances in the external environment or in the motor system itself [58]. This paradigm has extensively been used to study different aspects of motor learning using tasks such as force fields, prism goggles, and rotations. Visuomotor adaptation provides a behavioral method of intermediate complexity that combines low-level motor execution and high level of cognition [59], allowing us to assess short-term plasticity [60].

Current knowledge in motor control suggests that there are two learning processes driving motor adaptation: one implicit and the other explicit. Implicit learning is an automatic process where no awareness of that learning is taking place. Implicit learning occurs gradually. On the other hand, explicit learning uses strategic knowledge (cognitive strategies) for learning a particular task. This process allows to speed up learning.

2.7.1 Explicit and Implicit Mechanisms in Motor Adaptation

It was thought that visuomotor adaptation was mediated only by an implicit process that updates an internal model (forward model) that makes predictions of the sensory consequences of the motor commands. In a visuomotor rotation paradigm, subjects perform goal-directed movements under perturbed conditions they are not aware of. A perturbation of the visual feedback produces a mismatch between the visual consequences of the motor commands, while the proprioception consequences remain intact. This sensory prediction error is used to update the internal model. As subjects continuously practice, the sensory prediction error is reduced exponentially until the error is close to zero, converging to baseline levels. When the perturbation is removed, subjects make extensive errors in the opposite direction of the perturbation, which are called after-effects. The after-effects are considered proof that implicit learning was experienced.

However, recent evidence suggests that an explicit process also takes place in motor adaptation. When naive subjects experience a rotation for the first time, they observe their movements veering significantly off-target, and they might realize that there is some sort of perturbation affecting the task. This realization might lead them to use a compensatory strategy that allows them to perform similarly to baseline trials (no rotation). To study the explicit process, subjects were provided with a strategy to counteract the rotation. For instance, in [61], after experiencing 2 trials under rotated visual feedback, participants were provided with a strategy to counteract a CCW 45° rotation. When participants implemented the strategy, the directional error immediately decayed from 45° to near zero. However, this performance was not sustained for many trials, and as the experiment continues, the error increased, biased toward the direction that counteracts the rotation. Directional error across

trials during the rotation + strategy block showed that error evolved in an exponential decay fashion, as if subjects were adapting to the direction to counteract the rotation instead of the target. These results suggested that even though an explicit strategy successfully counteracts the rotation for a few trials, the implicit learning begins to operate but is not well calibrated. Instead of reducing the error between hand visual feedback and the target, it reduces the error between hand visual feedback and the direction used to counteract the rotation. By showing the conflict between implicit and explicit learning, this finding provides evidence that at least two different processes are involved in adaptation.

Evidence supporting the existence of these systems also comes from patients with cerebellar lesions. When the cerebellum is damaged, patients perform poorly, and deficits in learning are observed in adaptation tasks such as the visuomotor rotation and force field adaptation [62]. This suggests that implicit learning is associated with the cerebellum. Based on the findings reported in [61], patients with cerebellar lesions should be able to counteract a rotation task when provided with an explicit strategy, and their performance should be sustained and stable if their implicit system is impaired. Indeed, that is what was found in [63]. Patients with ataxia were able to counteract the rotation when a cognitive strategy was provided, and their performance was sustained compared to healthy control, which errors increased as shown in [61] due to implicit learning. These results support that implicit learning is linked to the cerebellum. In addition, it shows that by using a cognitive strategy, patients with ataxia and other cerebellar lesions can effectively counteract perturbation in a visuomotor adaptation task.

In the studies discussed previously, the strategy was explicitly given, allowing for the visualization of two conflicting systems. However, there was no task to dissociate implicit and explicit learning in adaptation where subjects were naive to the pertur-

bation. In [64], a novel version of the visuomotor rotation task was used to dissociate implicit and explicit learning. To this end, subjects were instructed to verbally report their aiming direction before reaching a target. A verbal report was considered a measure of the explicit process. Their findings suggest that both implicit and explicit components change across the entire learning process. The explicit process was responsible for the fast reduction of the error. On the other hand, the implicit process was slow and gradual as described by many authors.

2.7.2 Neural Substrates

The neural substrates involved in these processes are still under debate. However, there is substantial evidence that supports the involvement of the cerebellum in implicit learning. As mentioned previously, patients with cerebellar lesions were not able to learn a visuomotor rotation task [65]. Notably, when these patients were provided with an explicit strategy, they were able to counteract the perturbation and sustain performance [63]. In addition, neuromodulation techniques such as tDCS have been used to probe the function of the cerebellum in visuomotor learning tasks. For instance, in [66], anodal tDCS over the cerebellum, but not over the motor cortex, has shown an enhanced learning rate in a force field adaptation task. Similar results were shown in a visuomotor task [67].

On the other hand, explicit learning mechanisms are associated with the development of a cognitive strategy that allows to speed up learning. For this reason, explicit learning is associated with the Prefrontal Cortex (PFC), a brain region involved in high-level cognition, decision-making, and strategic learning. Using the visuomotor rotations task that dissociates implicit and explicit learning, described in [64], anodal tDCS over the dorsolateral PFC showed a small increase in explicit learning [68]. Also, a resting-state fMRI study showed that the frontoparietal resting-state network

and the cerebellum network were altered in the visuomotor rotation task [69]. The frontoparietal network is involved in attention, working memory, decision-making, and high-level processes. It connects areas such as the dorsolateral PFC and the posterior parietal cortex (PPC). In addition, a recent study showed that visuomotor impaired performance in older adults was associated with decreased gray matter volume in the hippocampus, medial temporal lobe, striatum, and prefrontal cortex [70]. Surprisingly, the cerebellum was not affected, suggesting that impaired performance in a visuomotor learning task in older adults is associated with explicit learning.

2.8 Discussion

In this literature review, we present the rationale for considering ONS and TNS as non-invasive alternatives to VNS, which could potentially enhance motor performance and serve as a non-invasive neuromodulation alternative for conventional rehabilitation. Although it is unknown whether TNS or ONS can have an effect in the motor domain, the vagus, trigeminal, and occipital nerves share a common pathway to neuromodulator centers in the brainstem, such as the LC, which projects across the brain and exerts its function through the release of NE. The LC-NE system is known to be involved in different brain functions such arousal, sensory-motor behavior, learning and memory [71], which are thought to be related to the therapeutic effects observed in VNS. Therefore, these similar anatomical projections might result in TNS and ONS also having an effect on motor behaviour, similar to VNS in stroke patients.

To test our hypothesis, we utilized a motor adaptation paradigm that has been extensively used in the domain of motor learning. As we reviewed, visuomotor adaptation involves two mechanisms: one explicit, leading to initial rapid learning, and the other implicit, responsible for gradual learning. Research suggests that several brain areas are involved in motor adaptation, with the cerebellum and prefrontal cortex

being linked to implicit and explicit learning, respectively. Studies using tDCs over the cerebellum and the dorsolateral PFC have demonstrated the ability to modulate the fast and slow learning process that drive motor adaptation [67, 66, 68]. Given that the LC projects to both of these areas, we proposed that neuromodulation using a bottom-up approach, such as TNS and ONS, might also have the potential to modulate the rate of learning in a visuomotor rotation task.

Chapter 3

METHODS

3.1 Introduction

This chapter will describe the common methods employed throughout Aims 1-3 conducted to investigate TNS effects on motor learning. Although each study exhibited variations in the experimental design (e.g., stimulation delivered offline v/s online, different stimulation parameters, etc.), the overall methodology employed was very similar. For instance, the conducted studies adhered to the same recruitment process, used the same task and apparatus for data collection, and shared data analysis procedures. Thus, this chapter will highlight all shared methods.

Specific details about each experiment will be provided in subsequent chapters, providing a more in-depth examination of the experimental variations and outcomes.

3.2 Subjects Recruitment

Right-handed individuals without prior history of neurological or psychiatric disorders (For more details see Appendix A) participated in this series of experiments. All potential candidates completed an online questionnaire (Google Forms) to determine eligibility for the study. Written consent was obtained from all participants in accordance with the Declaration of Helsinki. All subjects declared themselves as right-handed, but their handedness was also assessed using the Edinburgh Handedness Inventory-short form (For more details see Appendix B) [72]. All participants reported having normal or corrected-to-normal vision. Eligible individuals participated in only one experiment. Monetary compensation was provided to participants

for their involvement in a given study. All experimental protocols received approval from the Institutional Review Board at Arizona State University.

These studies were designed as single-blind randomized sham-controlled experiment. Block randomization was used to ensure equal sample sizes across groups using Sealed Enveloped ^{TM} [73].

3.3 Apparatus

Fig. 7.2 shows the setup used for these experiments. The apparatus was similar to the one used in [74]. Participants performed visually-guided reaching movements within a semi-immersive 3D virtual reality (VR) environment. A motion tracking system (Visualeyez VZ-3000; Phoenix Technologies, Inc.) was used to track arm movements. To this end, an LED sensor was attached to the right index finger of each participant. Visual images rendered in Vizard 3.0 (WorldViz Inc.) were displayed on a stereoscopic 3D monitor (Dimension Technologies Inc.) and projected onto a mirror that was embedded within a metal plate oriented at a 45° angle with respect to the monitor. During the experiment, participants were seated with their heads positioned on a chin rest and their eyes aligned with the center of the mirror. To prevent the arm from being directly viewed, movements were performed behind the metal plate, but visual feedback of the fingertip was provided to the participants in the form of a virtual cursor that was projected onto the mirror. Participants across all experiments used this apparatus and performed the task in the same room under the same light conditions.

3.4 Behavioral Paradigm

Participants performed a visuomotor rotation task, which requires gradually adapting to an imposed discrepancy between hand motion and corresponding visual feed-

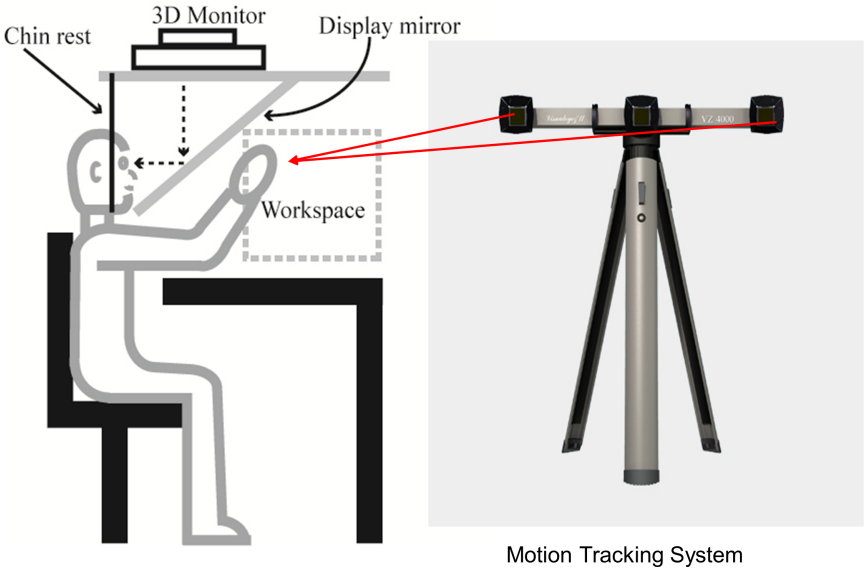

Figure 3.1: Semi-immersive 3D VR environment used during the experiments. Subjects were seated with their head positioned on a chinrest and their eyes aligned with the center of the mirror as their arm movements were captured by a motion tracking system.

back [59]. To this end, center-out reaching movements were made to eight targets, that appeared one at a time in a pseudorandom order, located 10 cm away from the starting position and 45° apart within a vertical plane (Fig. 3.2.A). Some cues that facilitated depth perception within the 3D environment were implemented. For instance, targets were surrounded by a yellow transparent sphere and appeared inside a cage (Fig. 3.2.B). Subjects were instructed to move as fast and accurately as possible and received visual feedback of their movements as well as auditory feedback for correctly hitting the target. To ensure that participants used approximately the same range of movement velocities, during the trial they also received visual feedback about movement speed and were encouraged to maintain a peak velocity that was greater than or equal to 0.5 m/s. Feedback about movement accuracy was also provided using a point-based scoring system designed to make the task more engaging. Additionally, participants were encouraged to take a break every 25 trials to amelio-

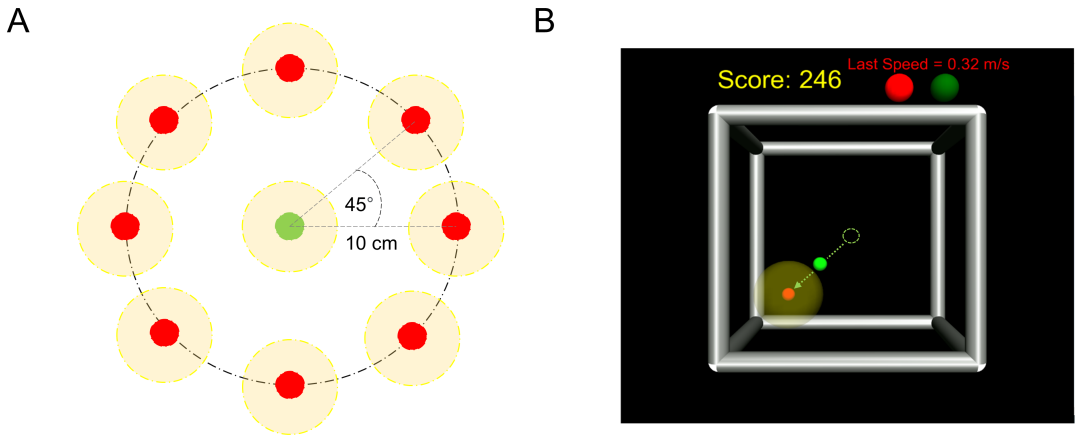

Figure 3.2: A) Location of targets relative to the starting position. B) 3D environment showing the hand position (green sphere), target (red sphere), cage, and performance feedback given to participants (top).

rate the fatigue in the right arm. To this end, a warning sign appeared on the screen with the message 'Take a break'. Resting periods could be more frequent if needed.

Although the specific design varied across the experiments, they all followed a similar structure. The study sessions began with a familiarization block, where participants were instructed on how to perform the task. Subsequently, the task progressed through the three main blocks of trials in a standard visuomotor rotation paradigm: Baseline, rotation, and washout [60].

Fig. 3.3 shows hand trajectories on a trial-by-trial basis and the expected error profile during a visuomotor task. During the baseline block, subjects performed reaching movements with veridical visual feedback, meaning their actual movements matched the visual feedback provided. As expected, arm trajectories were characterized by mostly straight paths to the targets with approximately zero error (Fig. 3.3.A). In the rotation block, which represented the learning phase of the experiment, participants encountered a 30° counterclockwise (CCW) rotation of hand visual feedback. This perturbation produced a mismatch between the actual movement and

the visual feedback, resulting in errors in initial movement direction. Hand trajectories pointed toward the cue target location during the early trials, but as the block progressed, trajectories gradually rotated toward the direction that counteracted the rotated visual feedback (Fig. 3.3.B). The directional error produced by the rotation decayed exponentially, converging toward baseline levels. Finally, in the washout block, the rotation of the visual feedback was removed. However, subjects still experienced a discrepancy between the movements and the visual feedback leading to errors in the opposite direction, known as 'aftereffects'. These aftereffect occurred due to the novel mapping between movement and visual feedback that was learned in the rotation block. Thus, in the washout block initial trajectories pointed toward the learned direction that counteracted the rotation in the previous block. After several movements were executed, hand trajectories gradually rotated toward the actual target direction, restoring the original mapping (Fig. 3.3.C). Thus, the error over time gradually decayed in an exponential fashion that converged to baseline levels. Note that participants did not receive any prior information about the introduction or removal of the rotated visual feedback, nor were they provided with strategies to counteract it.

The experiment was controlled using a custom program designed in LabVIEW (National Instruments Corp.) that allowed the record of kinematic data from the motion tracker and the export of the data into MATLAB files for further analysis. Additionally, the LabVIEW program controlled experimental parameters such as the number of trials, rotation angle, number of targets, etc., and provided the coordinates of the targets to be displayed in the 3D environment.

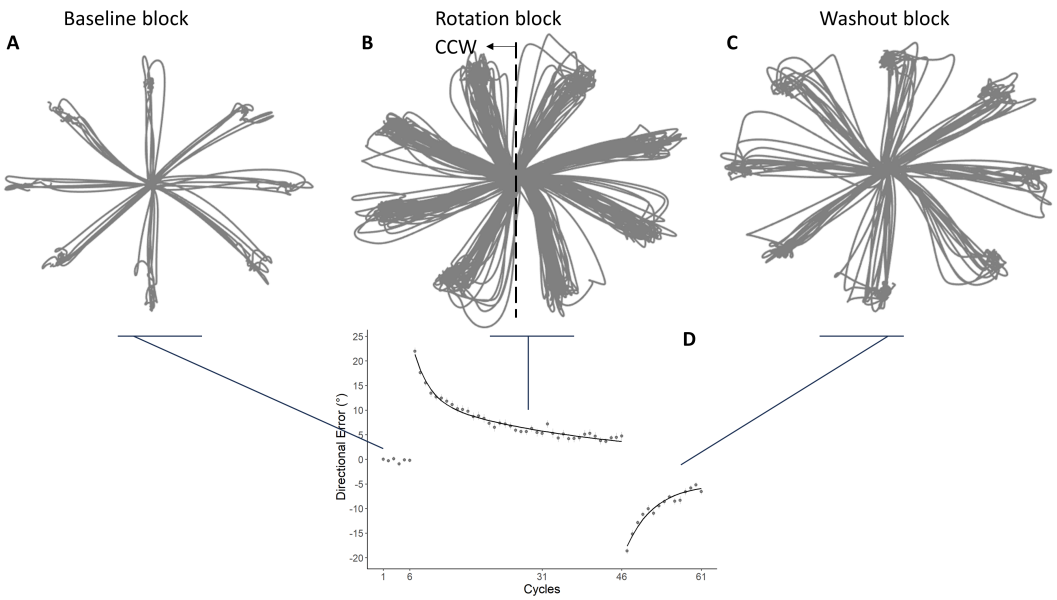

Figure 3.3: Characteristic trajectories during A) Baseline, B) Rotation, and C) Washout Block. D) Expected directional error profile during a visuomotor rotation task.

3.5 Stimulation Protocol

3.5.1 TNS intervention

TNS was applied using two round (diameter: 3.2 cm) surface electrodes (Axelgaard Manufacturing Co., Ltd.) placed on either side of the forehead to bilaterally stimulate the supraorbital branches of the trigeminal nerve [75]. Before the electrodes' placement, the forehead was cleaned using an alcohol prep pad to reduce the impedance between the electrode and the skin. The stimulation was delivered using a peripheral nerve stimulator approved for human research (DS8R, Digitimer Ltd.). The stimulator was triggered using a function generator (AFG 3022B, Tektronix Ltd.), controlled by a custom Graphic User Interphase (GUI) written in MATLAB that also allowed setting stimulation parameters such as the frequency, current, and pulse width, among others. Fig. 3.4 shows a biphasic symmetric square waveform used for TNS. Parameters such as pulse frequency and pulse width were different

depending on the corresponding experiment. The interphase pulse was set at 1 μs for all studies. All stimulation protocols were delivered in cycles of 30 seconds ON and 30 seconds OFF., similar to previous studies [23, 27, 26]. During the ON periods, the current was ramped up/down over 5 seconds to make the ON/OFF transitions more comfortable.

Before the stimulation session began, participants were told that a comfortable level of stimulation would be selected, and they might or might not feel it. Following this, the experimenter gradually increased the current until it reached either 1) a tolerable level that did not produce pain or discomfort or 2) an established maximum safety level. Once the intensity level was determined, it was used throughout the experiment, but participants could still request to reduce or stop the stimulation at any time.

3.5.2 Sham intervention

For the sham group, electrodes were placed in the same location as the experimental groups, but no stimulation was applied. However, the stimulator emitted the same clicking sounds as when the current was delivered. In all experiments, immediately after the stimulation session was completed (either active or sham), subjects were asked whether they felt the stimulation and, if so, the location of the sensations they experienced.

3.6 Post Study Survey

An online post-study survey was conducted using Google Forms to assess participants' overall experience during the study. The survey aimed to gather feedback on various aspects of the experiments such as discomfort level, side effects experienced, and attention level during the task.

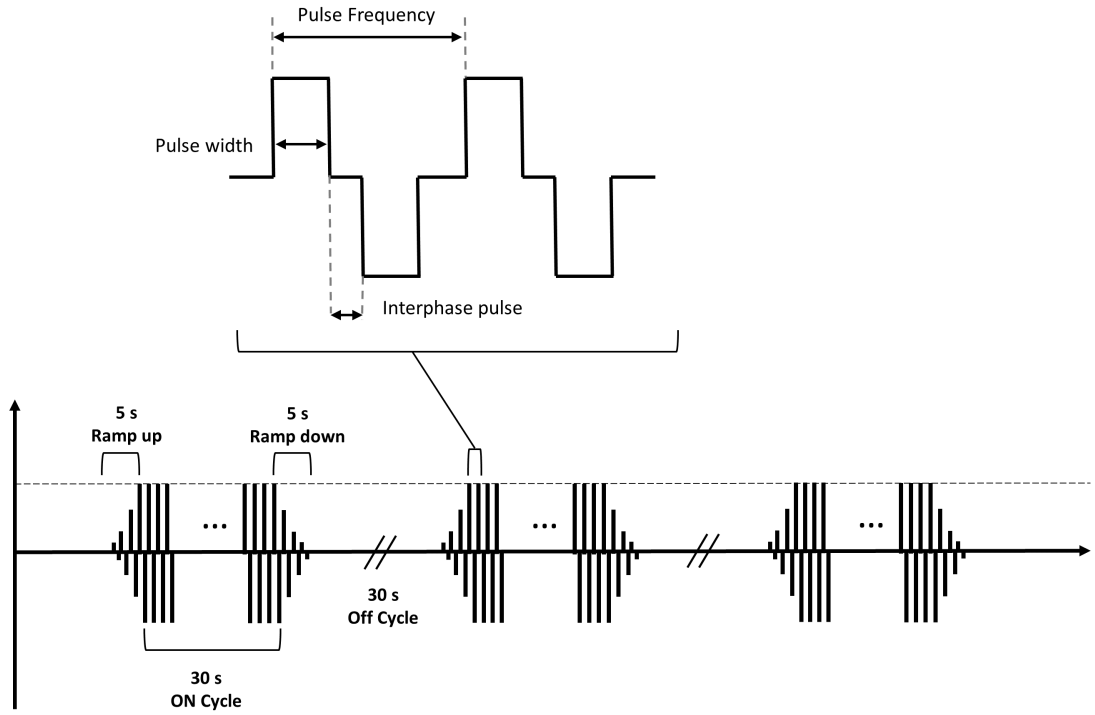

Figure 3.4: Stimulation parameters

Participants were inquired to rate their comfort level during the experiment on a scale of 1 to 10, with 1 indicating feeling uncomfortable and anxious, and 10 indicating feeling very relaxed. They were also requested to rate the level of discomfort or pain experienced during the experiment, if any, using a 1-10 scale. A rating of 1 represented barely noticeable discomfort, while a rating of 10 indicated unbearable discomfort.

Also, participants were asked about potential side effects observed in previous studies [23, 27] that the stimulation might evoke. Specifically, they were inquired about headaches, blurry vision, dizziness, and skin itching at the electrode site. If participants had experienced any of these effects, they were instructed to rate their discomfort on a scale of 1 to 10, where 1 represented barely noticeable discomfort, and 10 represented unbearable discomfort.

Finally, participants were also asked to rate their level of relaxation using a 1-10 scale, where 1 indicated mild relaxation, and 10 represented a deep trance-like or

meditative state. Similarly, they were inquired to rate their attention level during the task on a scale of 1 to 10, where 1 represented not paying attention and 10 represented being engaged and anticipating stimuli.

3.7 Data Analysis

Kinematic analyses were performed in MATLAB (MathWorks, Inc.). Hand movements were sampled at 125 Hz, filtered using a low-pass 2nd order Butterworth filter with a cutoff frequency of 6.25 Hz, and differentiated to obtain hand movement velocities. As shown in Fig. 3.5.A, tangential profiles were used to obtain parameters such as movement onset, Peak Velocity (PV), and Reaction Time (RT). Movement onset was estimated as the first point in time at which the movement exceeded 10% of the PV. The reaction time was measured from the moment the visual target was presented until movement onset. To determine the PV, movement onset, and RT, a semi-automatic script written in MATLAB was used. Each trial was reviewed, and if the movement onset was incorrectly detected, it was adjusted accordingly. Additionally, if the trial presented a velocity profile different from a typical bell shape or the movement trajectory did not follow a straight path, the trial was deleted. Data sets with more than 10% of the total movements deleted were not analyzed further.

Visuomotor performance was quantified by the Directional Error (DE), defined as the angular difference between the rotated visual feedback at PV and the target direction (see Fig. 3.5.B). Cycles were formed by binning 8 consecutive trials to each target direction. Within the cycles, any trial DE, PV, or RT exceeding two standard deviations from the mean was deleted. Subsequent analyses were performed on the binned cycle data.

Individual directional errors during the rotation and washout block were corrected based on the intrinsic bias observed during baseline. Intrinsic bias was calculated

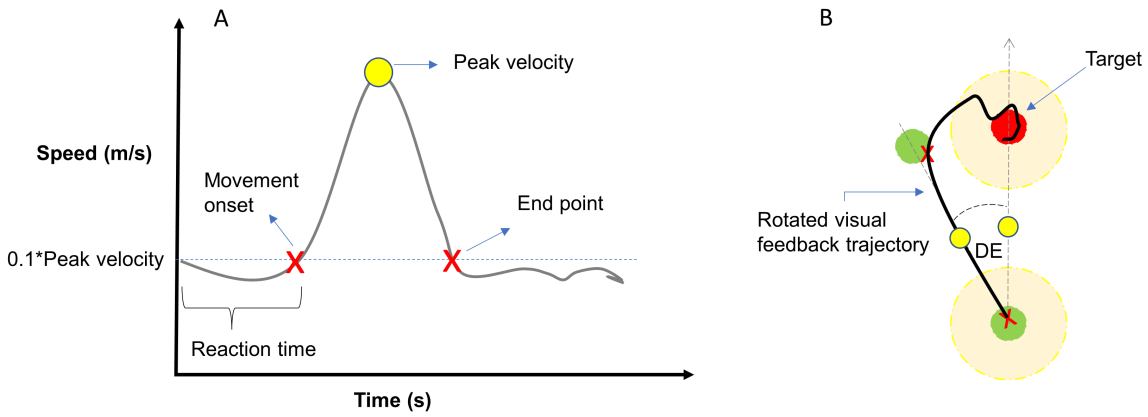

Figure 3.5: A) Parameters obtained from velocity profile. B) Illustration of directional error

based on the procedure described in [76]. To this end, we first calculated the mean directional error during baseline for each direction. Subsequently, these biases were subtracted from the DE of each direction during the rotation and washout blocks.

Statistical analyses were performed in R (R Core Team, 2014). Following the convention of several previous studies, [77, 78, 79], participant's mean DEs during the rotation block were fitted to a double exponential model (see Eq. 1). This allowed for quantification of the fast and slow learning processes that drive motor adaptation. To this end, a nonlinear least squares procedure based on the Levenberg-Marquardt algorithm was used (nlsLM function in R) to fit the following model:

$$\hat{DE}_r = C_1 e^{-\alpha * i} + C_2 e^{-\beta * i} \tag{1}$$

where, $\hat{DE}_r$ is the estimated directional error during the rotation block, α and β represent the fast and slow learning rate, C_1 and C_2 are the magnitudes of each exponential and i is the i-th cycle during the rotation block. We assumed that α and $\beta > 0$, $\alpha > \beta$ and C_1 and $C_2 > 0$.

On the other hand, mean DEs for the washout block were fitted to the following

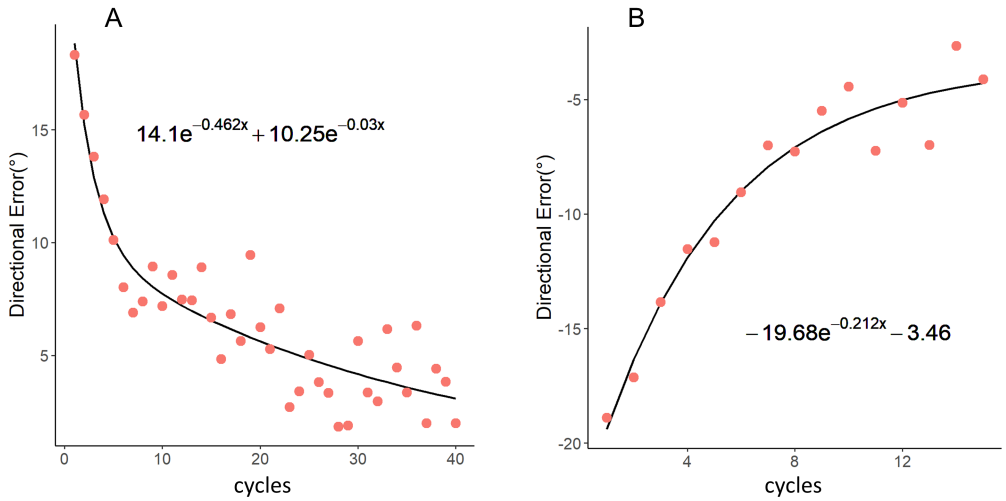

Figure 3.6: A) Directional error during the rotation block fitted to a double exponential model. B) DE during the washout block fitted to a single exponential model.

single exponential model:

$$\hat{DE_w} = Ae^{-\gamma * i} + C \tag{2}$$

where, $\hat{DE_w}$ is the estimated directional error during the washout block, γ represents the forgetting rate, A is the magnitude of the exponential, i is i-th cycle during the washout block and C is a constant. We assumed that $A < 0$ (Error in the opposite direction) and $\gamma > 0$.

Fig. 3.6 shows DEs from a representative subject during the rotation (A) and washout (B) blocks. A double and a single exponential model were fitted to the rotation and washout block, respectively.

To compare the learning and forgetting rates across different groups, various statistical tests were used based on the distribution of the data. Parametric or non-parametric tests were chosen depending on the presence or absence of normality in the data. Normality was assessed using the Shapiro-Wilk test, and further verified by examining Q-Q plots. In some cases, bootstrap and permutation tests were utilized to augment these analyses.

Changes in RT and PV were assessed throughout the rotation block, focusing on

early and late adaptation phases. To estimate early adaptation, the average RT and PV across the first 15 cycles were calculated for each subject's dataset. Similarly, to estimate late adaptation, the mean values across the last 15 cycles were computed. Comparisons were performed using a two-factor mixed ANOVA (between-subjects factor: experimental groups; within-subjects factor: early and late adaptation phase). A similar approach was used for the washout block. The early phase of the de-adaptation was estimated using the average of the first 6 cycles, while the late phase was estimated based on the mean of the last 6 cycles. A two-factor mixed ANOVA was used on the averaged data (between-subjects factor: experimental groups; within-subjects factor: early and late de-adaptation phase). All p-values were adjusted using the Bonferroni correction.

Correlations between learning rate coefficients and attention levels obtained from the post study survey were also explored, as were correlations between the rate coefficients and the amounts of current received.

For all statistical tests, a p-value < 0.05 was considered as significant.

Chapter 4

EFFECTS OF OFFLINE TNS ON VISUOMOTOR LEARNING

4.1 Introduction

Most of the frequencies ranges used in electrical stimulation of the nervous system are below 500 Hz [80]. Trigeminal Nerve Stimulation (TNS) is not the exception. Frequencies ranging from 60-120 Hz are commonly found in TNS literature. For instance, TNS at 60 Hz has shown therapeutic effects on migraine [28]. On the other hand, TNS applied at 120 Hz has been used in clinical trials for Drug-Resistant Epilepsy (DRE), depression, Attention-Deficit/Hyperactivity Disorder (ADHD), among others conditions [23, 27, 26]. Thus, these clinically tested protocols serve as valuable starting point to assess the potential of TNS in the context of motor learning.

Additionally, recent efforts aimed at optimizing electrical stimulation parameters in other contexts have led some investigators to explore supraphysiological frequencies in the kilohertz range. For instance, transcutaneous Kilohertz Electrical Stimulation (KES) of the trigeminal and cervical spinal afferents was shown to facilitate relaxation, dampen sympathetic responses to acute stress, and improve sleep quality and mood in healthy adults [7, 50]. Interestingly, the KES protocols used in these studies allowed to deliver higher current intensities relative to others modalities such as Transcranial Direct Current Stimulation (tDCS) and Transcranial Alternating Current Stimulation (tACS) with more tolerable skin sensations, which was associated to less motor and pain fibers activation. Also, transcutaneous KES have been explored as a means to enhance proprioceptive sensitivity and learning [81, 57]. These studies provide encouraging data on the potential use of KES to modulate behaviour via the

trigeminal nerve.

This chapter discusses the findings of two pilot studies that aimed to assess the effects of single-session offline TNS on motor learning in healthy adults. Each study focused on investigating a specific stimulation protocol. In experiment 1A, clinically tested frequencies, i.e., 120 and 60 Hz, were used. In experiment 1B, a novel 3 kHz TNS protocol was used. The chapter characterizes the TNS protocols and their effects on visuomotor learning, and therefore addresses Aim 1, where we hypothesized that the groups receiving TNS would experience learning enhancement in a visuomotor rotation task.

4.2 Participants

4.2.1 Experiment 1A: Offline TNS Using 120 Hz and 60 Hz

A total of sixty-seven (67) participants were recruited for this study. All of them were self-reported as right-handed however three participants were classified as mixed-handed according to the Edinburgh Handedness Inventory. Two of them scored 50, and one scored 37.5, which is below the threshold of 62.5 for right-handedness. Despite this classification, all three participants were included in the analysis. Four data sets were discarded: one (1) participant abandoned in the middle of the experiment due to a conflict in her schedule, and three (3) subjects had noisy kinematic data, resulting in the removal of over 10% of the movements. As described in 3.7, subjects whose data had more than 10% of the movements removed during the data cleaning process were not included in the analysis. The remaining sixty-three (63) young adults (18 - 36 y/o; 22.75 $\pm$ 4.6 y/o; 32 female and 31 men) were randomly assigned to one of three different groups: 120 Hz, 60 Hz, and sham.

4.2.2 Experiment 1B: Offline TNS Using 3 kHz

A different cohort of forty-three (43) healthy participants was recruited for this study. Subjects were self-reported and classified as right-handed by the Edinburgh Handedness Inventory (score $\geq$ 62.5). Data from one subject was discarded because she was not capable to complete the experiment due to light-headiness after receiving 3 minutes of stimulation. She had fainted once in the past and had a family history of fainting which was not reported, even though the consent form and eligibility criteria survey explicitly asked for it. The participant recovered fully after stopping the stimulation. The remaining 42 subjects (18 - 40 y/o; 23.4 $\pm$ 4.6 y/o; 10 female and 32 male) were randomly assigned to one of two different groups: 3 kHz stimulation and sham.

4.3 Experimental Procedure

Fig.4.1 shows the experimental design for experiments 1A and 1B. Participants performed a visuomotor rotation task using the apparatus described in 3.3. The experiment began with a familiarization block, followed by one baseline block of 48 trials performed with veridical visual feedback. After the baseline block, subjects received either TNS or sham for 20 minutes under resting conditions. During this period, they viewed images of neutral valence and arousal (131 images with mean valence scores: 5.17$\pm$0.4 and mean arousal scores: 3.36$\pm$0.76) obtained from the International Affective Picture System (IAPS), in order to minimize variations in emotional states across participants [82]. Each image was displayed for 5 seconds followed by a black screen with a white crosshair in the center for 4.2 seconds. Once the stimulation session was completed, subjects were asked to mark in a diagram of the face anatomy where they felt the stimulation. Immediately after, a second baseline block of 48 trials

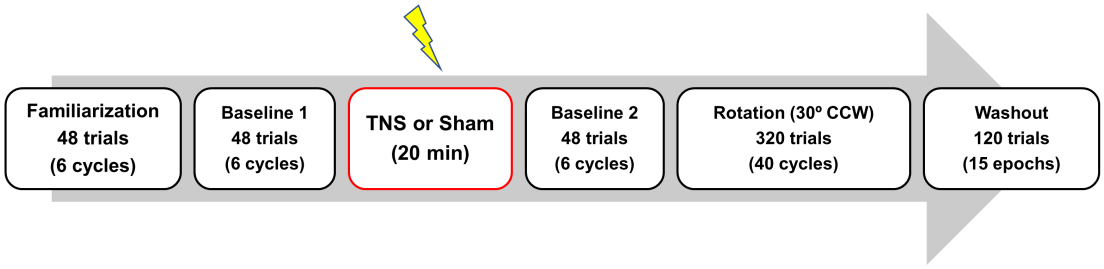

Figure 4.1: Experimental design for offline TNS experiments.

with veridical feedback and no stimulation was performed to determine whether the stimulation affects baseline motor performance. After the second baseline block, a single adaptation block of 320 trials involving a 30° CCW rotation of the hand visual feedback and no stimulation was performed. The experiment ended with a washout block of 120 trials, where the rotation was removed, and no stimulation was applied.

4.4 Stimulation Parameters

The overall stimulation protocol for experiments 1A and 1B was previously described in 3.5. Regarding the stimulation parameters, the frequencies under study in experiment 1A were 60 and 120 Hz, both with a pulse width of 250 μs. On the other hand, for experiment 1B, the stimulation was delivered at 3 kHz with a pulse width of 50 μs.

Stimulation over the supraorbital branches of the trigeminal nerve was delivered in cycles of 30 seconds ON and 30 seconds OFF for 20 minutes in both experiments. Therefore, participants received 10 minutes of effective TNS. The current applied was self-determined by each individual and set to a submaximal tolerable threshold. For the sham groups, electrodes were located on the forehead like those of the active groups, but they did not deliver any current.

4.5 Post Study Survey

Questions regarding attention level, side effects, and discomfort described in 3.6 were applied to participants after the experiment. In addition, participants were inquired about the content of the images displayed during the stimulation. To this end, they rated the content of the images in terms of how it made them feel, using a 1-10 scale, where 1 indicated unpleasant, 5 was neutral, and 10 was pleasant.

4.6 Data Analysis

4.6.1 Analyses of Baseline Performance

In experiments 1A and 1B, we conducted a two-factor mixed ANOVA to examine the effects of the experimental groups (between-subjects factor) and pre/post-stimulation (within-subjects factor) on baseline performance. For each subject's data set, the mean Reaction Time (RT), Peak Velocity (PV), and Directional Error (DE) across cycles were calculated separately for baseline 1 and 2 and analyzed statistically.

4.6.2 Analyses of Adaptation and Post-Adaptation Performance

During the rotation and washout blocks, RT and PV were compared between early and late adaptation and deadaptation phase using a two-factor mixed ANOVA as described in section 3.7.

Changes in DE during the rotation and washout block involved different statistical tests for each experiment. For instance, in experiment 1A, α, β, and γ coefficients were not normally distributed (Shapiro-Wilk test, $p < 0.05$). For this reason, the non-parametric Kruskal-Wallis test was used. Pairwise multiple comparisons using the Dunn's test were performed if significant results were found. P-values were adjusted using the Benjamini-Hochberg correction.

For experiment 1B, the distribution of the α and γ coefficients did not meet the criteria for normality based on the Shapiro-Wilk test ($p < 0.05$). As a result, the Wilcoxon rank-sum test was used for these coefficients. On the other hand, β coefficients were not different from a normal distribution (Shapiro-Wilk test, $p < 0.05$). Hence, a T-test was used.

4.7 Results

4.7.1 Experiment 1A: Offline TNS Using 120 Hz and 60 Hz

On average, participants from the 120 Hz and 60 Hz groups received 3.1±0.8 mA and 3.4±0.8 mA, respectively. No differences were found between intensities among groups (T-test, t=-1.24, df=39.68, p=0.22). Men (n=18, 3.7±0.7 mA) were able to tolerate higher stimulation intensities (T-test, t=-3.6, df=37.7, p=0.00091) than women (n=24, 2.9±0.7 mA).

Reaction Time

Fig. 4.2 shows the reaction times for each group throughout the experiment. This figure shows that reaction times fluctuated somewhat from cycle to cycle but did not appear to consistently increase or decrease within or across epochs. In baseline 1, the average reaction time for each group was distributed as follows: 120 Hz (348.8±23.9 ms), 60 Hz (344.5±38.2 ms), and sham (339.8±30.4 ms). In baseline 2, all groups showed a slight reduction in RT. The average RT had the following distribution among groups: 120 Hz (341.3±26.9 ms), 60 Hz (339.2±45.6 ms), and sham (337.5 ±30.7 ms). A two-factor mixed ANOVA was used to assess the effects of groups and pre/post-stimulation on RT. This analysis showed that neither groups (F=0.21, p=0.81) nor pre/post-stimulation baselines (F=3.82, p=0.055) had a statistically significant effect

on RTs. This two-factor ANOVA revealed that there was not a statistically significant interaction between group and pre/post-stimulation (F=0.36, p=0.701)

During the early adaptation phase (cycles 1-15), the mean reaction time for each group was as follows: 120 Hz (342.0±23.9 ms), 60 Hz (348.4±39.7 ms), and sham (336.0±25.9 ms). In the late adaptation (cycles 25-40), mean RT was the following: 120 Hz (338.6±26.1 ms), 60 Hz (343.0±39.6 ms), and sham (334.4±29.8 ms). A two-factor mixed ANOVA showed no statistically significant differences main effects of groups (F=0.61, p=0.545) or early/late adaptation (F=3.41, p=0.07) on RT. Additionally, no interaction was found between group and early/late adaptation (F=0.34, p=0.72)

Finally, for the early washout phase (cycles 1-6), the mean RT was as follows: 120 Hz (348.8±23.9 ms), 60 Hz (344.5±38.2 ms) and sham (339.8±30.4 ms). In the late phase (cycles 9-15), participants presented the following mean RT: 120 Hz (341.5±26.9 ms), 60 Hz (339.2±45.6 ms), and sham (337.5±30.7 ms). A two-factor mixed ANOVA determined that there was no statistically significant main effects of groups (F=0.85, p=0.432) or early/late deadaptation phases (F=0.27, p=0.602) on RT. There was no statistically significant interaction between group and early/late deadaptation.

Peak Velocity

Fig. 4.3 shows the peak velocities for all groups across the experiment. During baseline 1, the mean PV per group was as follows: 120 Hz (0.50 ±0.13 m/s), 60 Hz (0.51±0.1 m/s), and sham (0.46±0.098 m/s). In baseline 2, peak velocities tend to increase for all groups. The average PV was distributed as follows: 120 Hz (0.52 ±0.15 m/s), 60 Hz (0.55±0.12 m/s), and sham (0.51±0.13 m/s). A two-factor mixed ANOVA showed no statistically significant main effects of groups on mean PV (F=0.8,

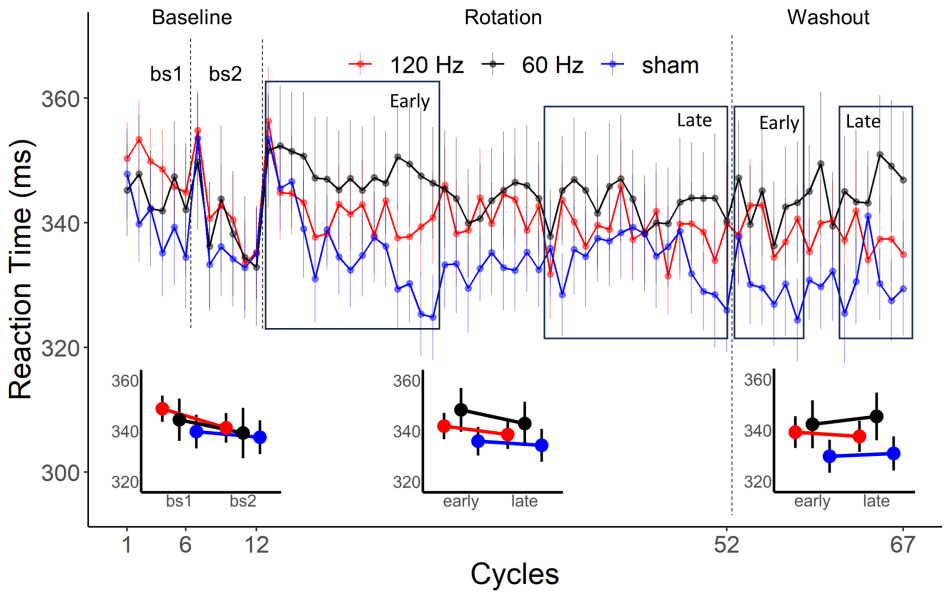

Figure 4.2: Mean reaction time (±SEM) for the 120 Hz (red), 60 Hz (black) and sham (blue) groups across experiment 1A.

p=0.452), and there was a statistically significant main effect of pre/post-stimulation on mean PV (F=21.99, p<0.001). A pairwise comparison shows that the mean PV of the 60 Hz (p=0.005) and sham (p=0.005) groups were statistically significant between pre/post-stimulation. This two-factor ANOVA also revealed that there was not a statistically significant interaction between groups and pre/post- stimulation (F=1.585, p=0.213).

The mean PV for all groups observed an increasing trend from early to late adaptation. During the early phase of the rotation block, the mean PV for each group was as follows: 120 Hz (0.50±0.1 m/s), 60 Hz (0.51±0.1 m/s), and sham (0.48±0.1 m/s). In the late adaptation phase, the mean PV increased for all groups: 120 Hz (0.53±0.1 m/s), 60 Hz (0.57±0.1 m/s), and sham (0.52±0.1 m/s). A two-factor mixed ANOVA showed no statistically significant (F=0.78, p=0.463) main effect of groups on PV. There was a statistically significant effect of early/late adaptation on PV (F=39.57, p<0.001). The pairwise comparison revealed that the mean PV of all

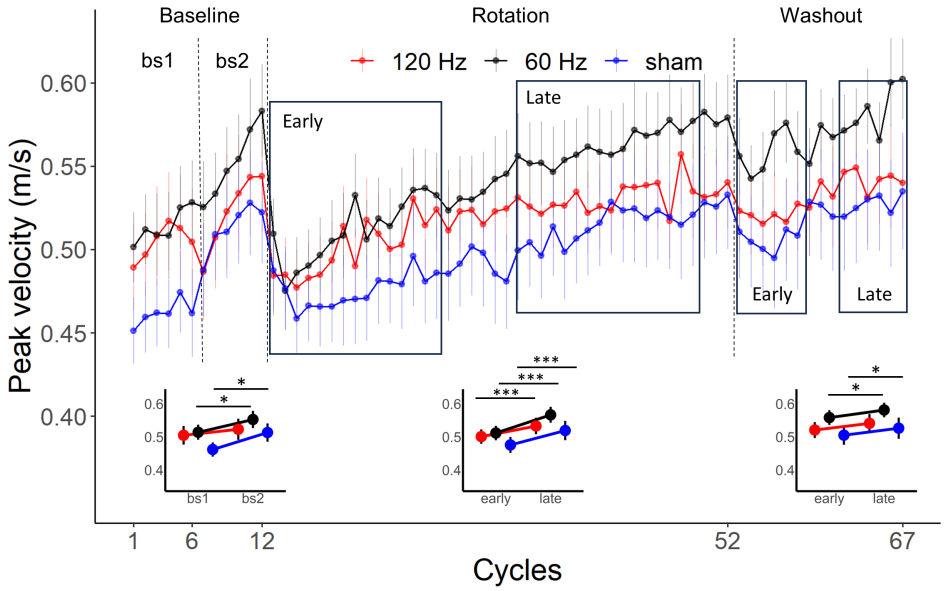

Figure 4.3: Mean peak velocity (±SEM) for the 120 Hz (red), 60 Hz (black) and sham (blue) groups across experiment 1A.

groups were statistically significant between early/late adaptation (120 Hz: p=0.006; 60 Hz: p<0.001; sham: p=0.024). The two-factor revealed no interaction between groups and early/late adaptation (F=0.92, p=0.401).

Lastly, in the early deadaptation, the mean PV was as follows: 120 Hz (0.52±0.11 m/s), 60 Hz (0.56±0.10 m/s), and sham (0.51±0.13 m/s). For the late deadaptation, the mean PV per group was the following: 120 Hz (0.54±0.13 m/s), 60 Hz (0.58±0.10 m/s), and sham (0.53±0.15 m/s). Two-factor mixed ANOVA showed no statistically significant main effect of groups on PV (F=1.15, p=0.32). There was a statistically significant main effect of early/late deadaptation on PV(F=13.54, $p < 0.001$). Pair-wise comparison showed a statistically significant difference in PV for 60 Hz (p=0.038) and sham (p=0.025) groups between early and late deadaptation. No interaction was found between group and early/late deadaptation (F=0.019, F=0.98).

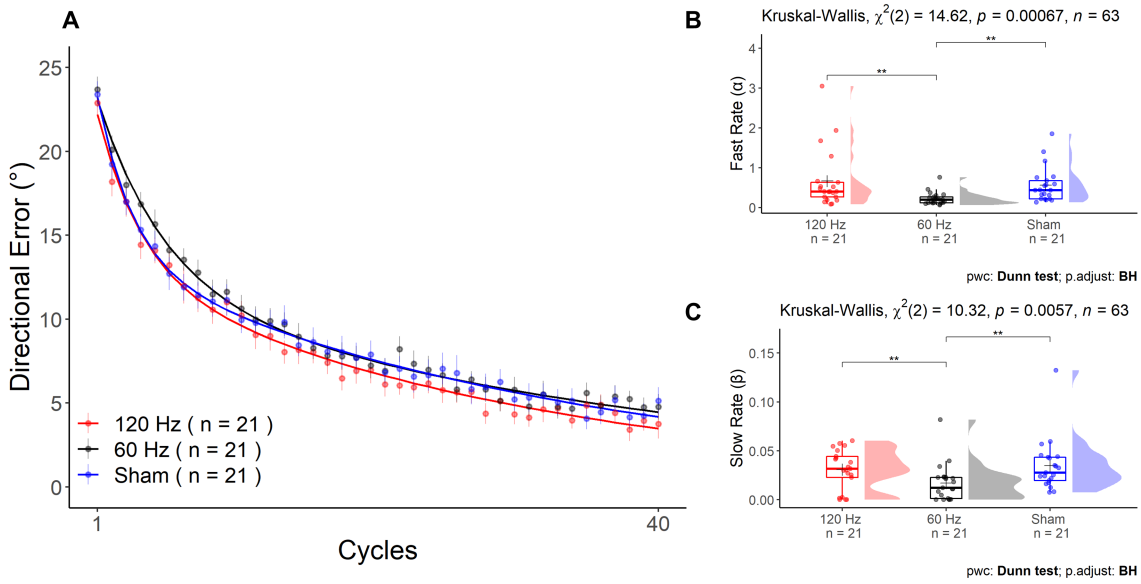

Figure 4.4: A) Learning curves for the 120 Hz (red), 60 Hz (black) and sham (blue). B) Fast and C) slow rates obtained from a double exponential model fitted to the average DE data for each group.

Directional Error

Directional error during baseline 1 were close to 0° for all groups (120 Hz: 0.1°±1.3, 60 Hz: -0.09°±1.3, Sham: -0.1°±1.3) and during baseline 2 (post-stimulation) DEs were also negligible (120 Hz: 0.3°±1.6, 60 Hz: -0.09°±1.6, Sham: -0.1°±1.3). A two-factor mixed ANOVA was used to assess the effects of groups and pre/post-stimulation on DE. This analysis showed that neither of groups (F=0.36, p=0.697) nor pre/post-stimulation (F=0.29, p=0.595) had statistically significant effects on DE. Also there was not statistically significant interaction between groups and pre/post- stimulation (F=0.08, p=0.926).

Visuomotor performance (i.e., DE) throughout the rotation block for all groups is illustrated in the form of learning curves in Fig. 4.4.A. Visual inspection of these curves suggests that the 60 Hz group (black bold line) had the slowest adaptation. Analysis of the corresponding learning rates (Fig. 4.4.B and C) revealed a significant

difference between groups when fast and slow rates obtained from the double exponential model were analyzed (Kruskal-Wallis test; α: χ^2=14.62, df=2, p=0.00067; β: χ^2=10.32, df=2, p=0.0057). A post hoc pairwise comparison using the Dunn test revealed that both the fast and slow rates from the 60 Hz group were significantly different from those of the 120 Hz (α: p=0.0019, β: p=0.00065) and sham groups (α: p=0.00049, β: p=0.00045). On the other hand, as shown in Fig. 4.4.A, learning curves were initially similar between the 120 Hz (red bold line) and sham (blue bold line) groups but gradually diverged, with the 120 Hz group exhibiting slightly smaller DEs than sham later in the adaptation block. However, the fast and slow rates comparison showed no statistically significant differences between 120 Hz and Sham (α: p=0.705, β: p=0.906).

Lastly, Fig. 4.5.A shows the directional error during the washout block. All groups experienced aftereffects, i.e., DEs in the opposite direction reflecting participants learning. A visual inspection of Fig. 4.5.B suggests that the sham group demonstrated faster forgetting rates. However, rate coefficients were highly variable across subjects and as results the rate at which the DEs decayed was similar between groups (Kruskal-Wallis test, χ^2=4.55, df = 2, p=0.1).

Sham Analysis

For the sham group, electrodes were placed in the same location as active groups, but no current was applied. After the sham stimulation session, participants were asked to indicate on a diagram of the face where they perceived sensations. Five (5) out of twenty-one (21) subjects from the sham group reported having experienced sensations consistent with the stimulation. As a result, we explored how the perception of received stimulation affected learning rates. To this end, the sham group was split into subjects who perceived the stimulation (***Sham-felt***; n=5) and who did not

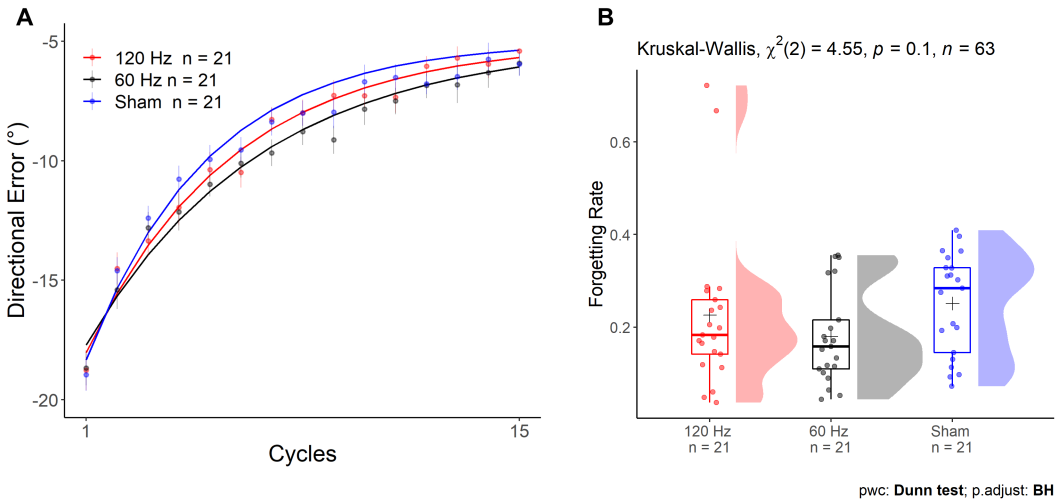

Figure 4.5: A) Directional error during washout block fitted to a single-exponential model. B) Forgetting rates were not significantly different between groups

(***Sham-no***; n=16). Interestingly, as shown in Fig. 4.6, the ***Sham-felt*** subjects learned the rotation at a similar rate as those receiving 120 Hz stimulation, which trended toward faster learning. On the contrary, the ***Sham-no*** subjects initially performed in a manner similar to the 120 Hz subjects but later converged toward learning rates consistent with 60 Hz stimulation.

Post Study Survey

Sixty (60) out of 63 subjects completed the post-study survey. The level of attention reported suggests that all groups were engaged in the task (120 Hz: 7.8/10 ±2.2, 60 Hz: 8/10 ±1.8, sham: 7.8/10 ±2.3). In the 120 Hz, a positive correlation was observed between the attention level and the α coefficient (R=0.48, p=0.032). No other statistically significant correlations between attention level received and the learning rates were found.

A small group of participants, specifically 10% (6 out of 60) experienced some level of discomfort during the experiment. On average, the discomfort was rated as mild, with an average score of 3.8±2.2 on a scale of 1-10, where 1 represents

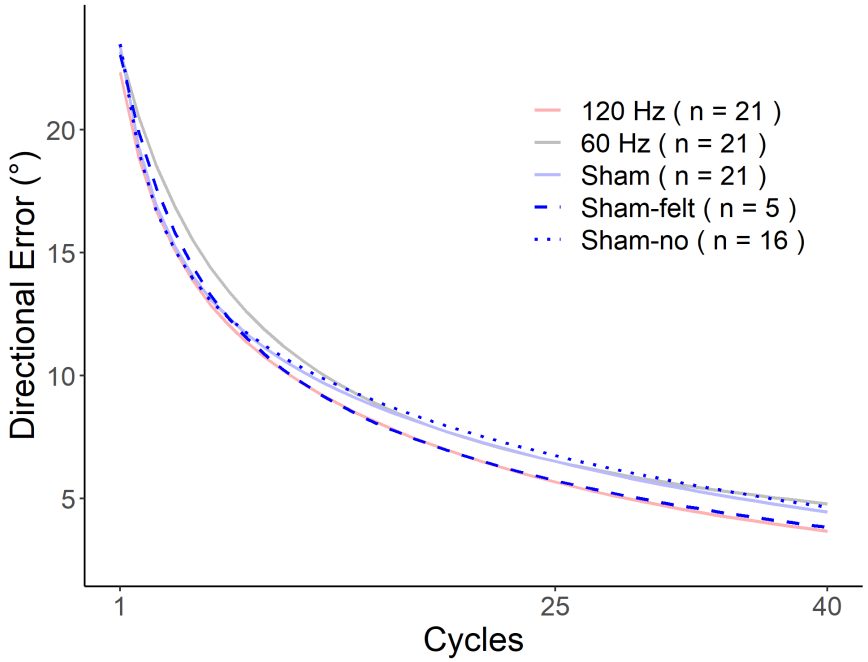

Figure 4.6: Learning curve for the sham group was split into participants who experienced the stimulation (Sham-felt, dashed blue line) and who did not (Sham-no, dotted blue line)

barely noticeable discomfort and 10 signifies unbearable pain. When examining the individual groups, at least one participant in each experimental group experienced discomfort (120 Hz: n=1, score=4/10; 60 Hz: n=3, score=3/10 ±1; sham: n=2, score=5/10 ±4.2).

Fig. 4.7 shows the distribution among groups when consulted for specific side effects they might have experienced during the experiment. This figure indicates that 10% or fewer participants in each groups report blurred vision (120 Hz: n=2, score=5.5/10 ±3.5; 60 Hz: n=2, score=3.5/10 ±1.4; sham: n=1, score=4/10), and approximately 15% or fewer experienced a headache (120 Hz: n=1, score=3/10; 60 Hz: n=3, score=4.3/10 ±2.5; sham: n=2, score=5/10 ±1.4). Only 10% of subjects in the sham group (but no other group) reported dizziness (sham: n=2, score=4.5/10 ±0.7). Finally, skin itching at the electrode site occurred in fewer than 10%, of participants in the stimulation groups (120 Hz: n=2, score=3/10 ±1.4; 60 Hz: n=1,

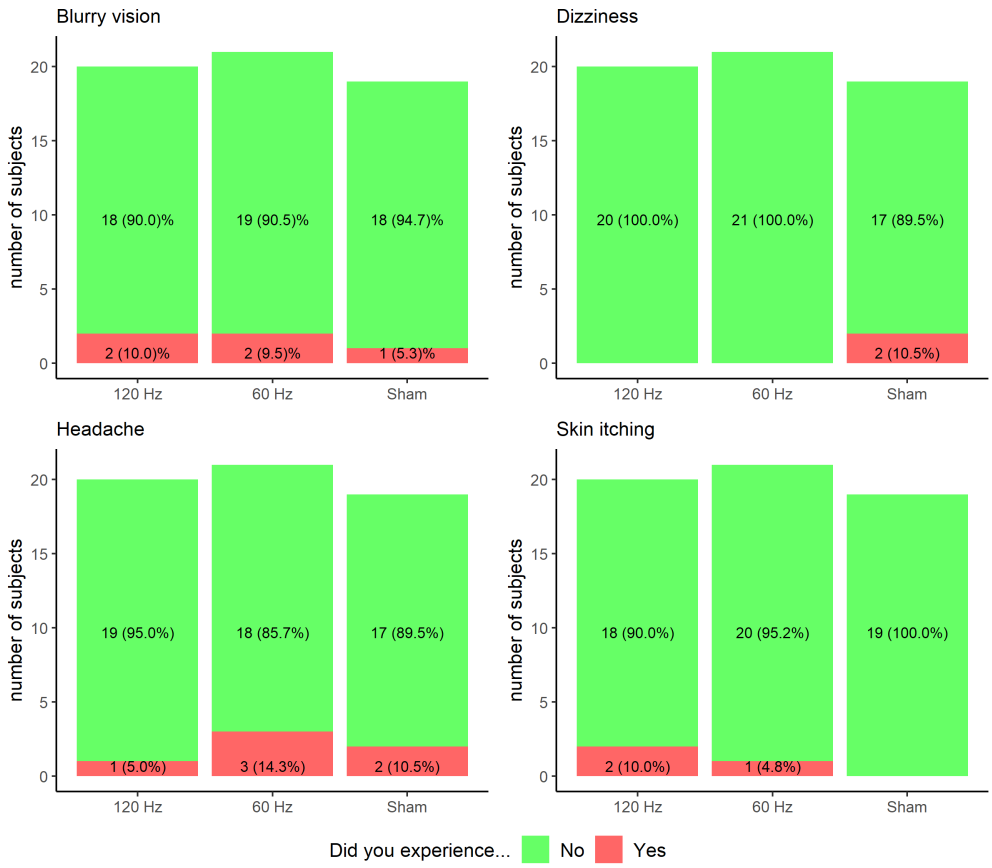

Figure 4.7: Side effects reported during experiment 1A. Specifically, subjects were consulted for blurry vision, headache, dizziness, and skin itching at the electrode site.

score=6/10), and in zero participants in the sham group.

4.7.2 Experiment 1B: Offline TNS Using 3 kHz

Participants in the 3 kHz group received a mean current of 8.4±1.8 mA during 20 minutes of stimulation. Men (n=17, 8.5±1.9 mA) and women (n=4, 7.9±1.5 mA) within this group were able to tolerate similar intensity levels (Welch test, t=-0.68, df=5.85, p=0.52).

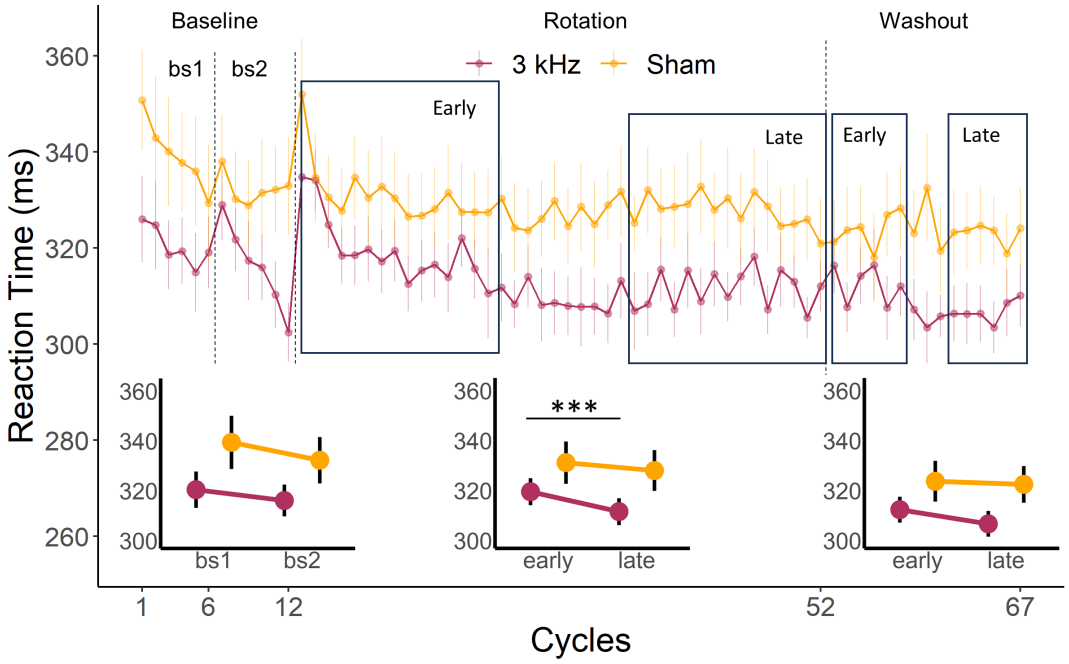

Figure 4.8: Mean reaction time (±SEM) for the 3 kHz (maroon) and sham (gold) groups across experiment 1B.

Reaction Time

Fig. 4.8 shows the RT for the 3 kHz and sham group across the experiment. The 3 kHz group showed slightly lower RT than sham in all blocks. During baseline 1, the mean RT was 320.4±33.5 ms and 339.5±48.9 ms for the 3 kHz and sham groups, respectively. In baseline 2, the average for each group was 316.1±29.2 ms for the 3 kHz and 332.3±42.7 ms for the sham. The two-factor mixed ANOVA showed no significant main effects of groups on RT ($F=2.21$, $p=0.145$). Although there was a statistically significant main effect of pre/post-stimulation on RT ($F=4.53$, $p=0.039$), the p-values corrected by Bonferroni during the pairwise comparison were not statistically significant for the 3 kHz ($p=0.233$) and sham ($p=0.096$). Additionally, the analysis showed that there was not a statistically significant interaction between groups and pre/post-stimulation ($F=0.285$, $p=0.596$).

During the early phase of the adaptation, participants in the 3 kHz group showed a mean reaction time of 319.6±24.7 ms. For the sham group, the mean RT was 331.2±38.9 ms. In the late phase, the 3 kHz and the sham groups presented a mean RT of 311.6±24.7 ms and 328.1±37.4 ms, respectively. A two-factor mixed ANOVA showed no statistically significant main effect of groups on RT ($F=2.09$, $p=0.156$). There was a statistically significant main effect of pre/post- stimulation on RT ($F=8.13$, $p=0.007$). A post hoc test revealed a statistically significant difference between the early and late adaptation phases for the 3 kHz group ($p<0.001$) but not for the sham ($p=0.373$). Also, there was no statistically significant interaction between groups and early/late adaptation ($F=1.52$, $p=0.225$).

Finally, mean RT during the early phase of the washout block were 312.4 ±23.8 ms for the 3 kHz group and 323.8±37.4 ms for the sham group. For the late phase, the mean RT was 306.7 ±23.6 ms for 3 kHz, and 322.5 ±33.6 ms for sham. A two-factor mixed ANOVA showed no statistically significant main effects of groups ($F=2.24$, $p=0.143$) or early/late deadaptation ($F=2.69$, $p=0.109$) on mean RT. This analysis also showed no statistically significant interaction between group and early/late deadaptation.

Peak Velocity

Fig. 4.9, shows the peak velocity for each group throughout the experiment. It is possible to observe that the PV for the 3 kHz group tended to increase as the experiment progressed. In baseline 1, the mean peak velocity was 0.45±0.09 m/s for the 3 kHz and 0.52±0.14 m/s for the sham. In baseline 2, the mean PV was 0.48±0.09 m/s and 0.55±0.15 m/s for the 3 kHz and sham, respectively. A two-factor mixed ANOVA showed no statistically significant main effects of groups ($F=4.06$, $p=0.051$) on PV. There was a statistically significant main effect of pre/post-stimulation ($F=11.24$,

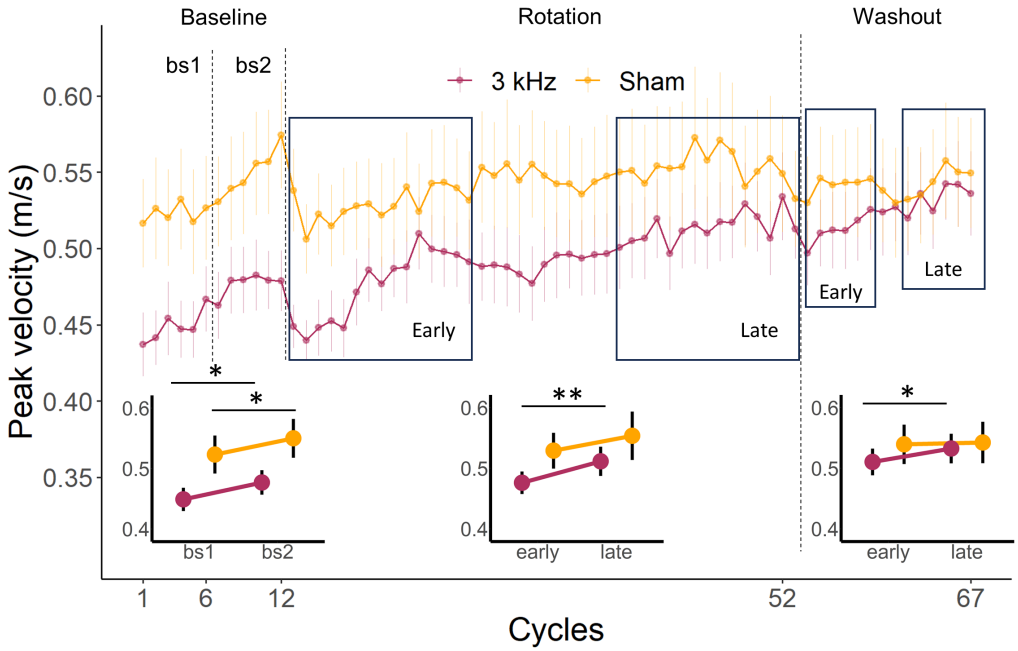

Figure 4.9: Mean peak velocities (±SEM) for the 3 kHz (maroon) and sham (gold) groups across experiment 1B.

p=0.002) on PV. A post hoc test revealed a statistically significantly different between pre and post-stimulation for both, the 3 kHz (p=0.016) and sham group (p=0.043). Additionally, this analysis also revealed that there was not a statistically significant interaction between group and pre/post-stimulation (F=0.006, p=0.940).

Mean peak velocities during the early phase of the rotation were 0.48±0.08 m/s and 0.53±0.14 m/s for the 3 kHz and sham, respectively. For the late phase, the mean PV for the 3 kHz group was 0.53±0.14 m/s and 0.55±0.18 m/s for sham. The two-factor mixed ANOVA analysis showed no statistically significant main effect of groups on PV (F=1.4, p=0.244). On the contrary, there was a statistically significant main effect of early/late adaptation (F=11.28, p=0.002) on PV. A post hoc multiple comparison revealed a statistically significant difference between early and late adaptation for 3 kHz (p=0.004) but no for the sham (p=0.099). Also, this analysis showed that there was not a statistically significant interaction between groups and

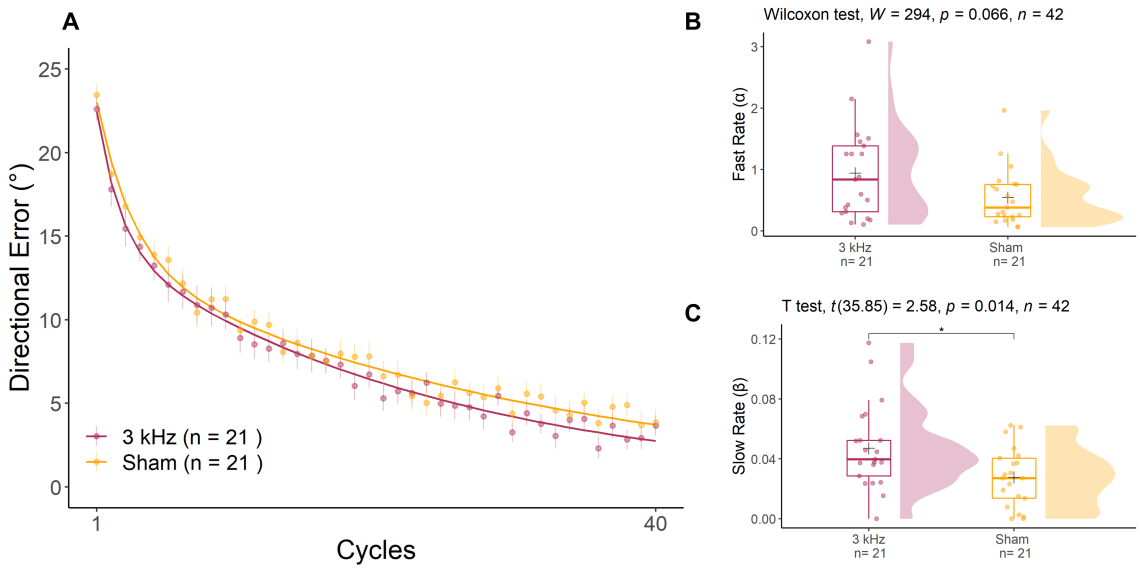

Figure 4.10: A) Learning curves for the 3 kHz (maroon) and sham (blue). B) Fast and C) slow rates obtained from a double exponential model fitted to the average DE data for each group.

early/phase adaptation on PV (F=0.366, p=0.549).

Mean peak velocities for the early deadaptation phase were 0.51±0.10 m/s and 0.54±0.15 m/s for the 3 kHz and the sham, respectively. In the late phase, mean PV were 0.53±0.11 m/s and 0.54±0.16 m/s for the 3 kHz and sham groups, respectively. The two-factor mixed ANOVA showed no statistically significant main effect of groups on PV (F=0.24, p=0.630). Also, there was a statistically significant effect of early/late deadaptation on PV (F=4.84, p=0.034). A post hoc pairwise comparison revealed a statistically significant between early and late deadaptation for the 3 kHz group (p=0.026) but not for the sham group (p=0.655). Also, the analysis showed no statistically significant interaction between group and early/late deadaptation (F=2.735, p=0.106).

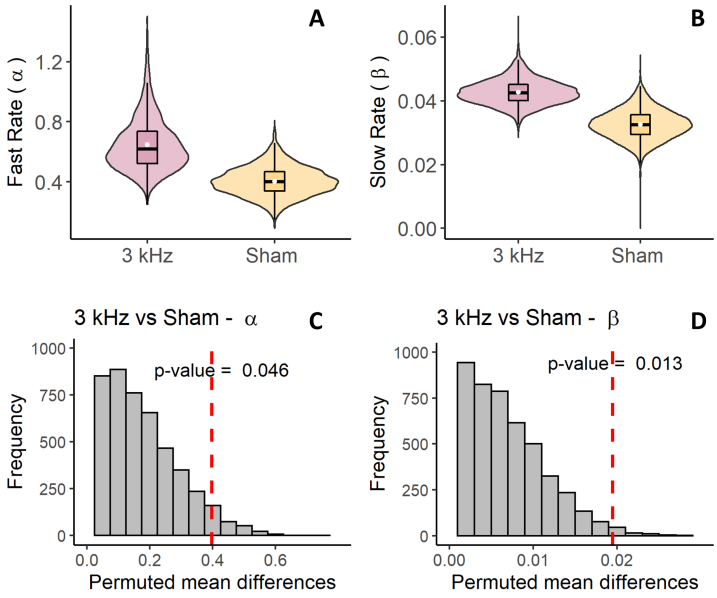

Figure 4.11: Bootstrap distributions (top A and B) and results of permutation tests (bottom C and D) performed on the fast and slow rate coefficients for experiment 1B.

Directional Error

Similar to experiment 1A, the DE among all groups was approximately zero during baseline 1 (3kHz: 0.38°±1.3, Sham: 0.25°±1.3) and baseline 2 (3kHz: 0.46°±1.4, Sham: 0.006°±1.4). A two-factor mixed ANOVA showed no statistically significant main effects of groups (F=0.56, p=0.458) or pre/post-stimulation (F=0.17, p=0.686) on DE.

Fig. 4.10.A shows the learning curves obtained after fitting a double exponential model to the average directional error for the 3 kHz (maroon bold line) and sham (orange bold line). The learning curve for the 3 kHz decayed faster than the sham, suggesting enhanced motor adaptation. Fig.4.10.B shows that the fast rate coefficients appeared to be greater for the 3 kHz than for the sham, with a tendency towards significance (Wilcoxon test, W=294, p=0.066). Similarly, Fig.4.10.C demonstrates that the slow rate coefficients were significantly greater for the 3 kHz compared to

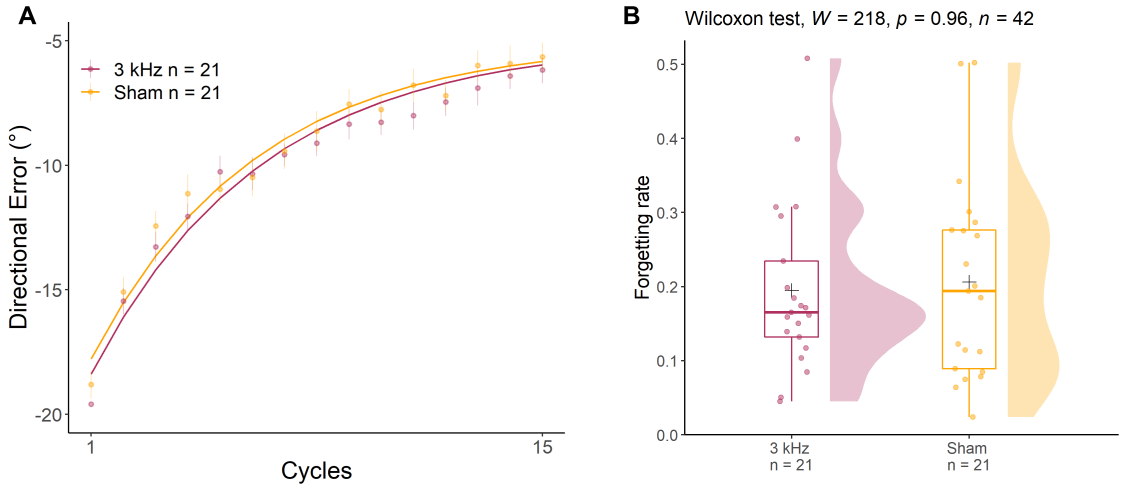

Figure 4.12: A) Directional error during the washout block. B) Forgetting rate.

the sham (T-test, t=2.58, p=0.014). Analysis of learning rates were consistent with the learning curves shown in Fig.4.10.A. Additionally, these results were in line with those provided by the permutation test and bootstrap resampling performed on the fast and slow rate coefficients shown in Fig.4.11.

Fig. 4.12.A shows the directional error fitted to a single exponential model during the washout block. Both groups experienced aftereffects, i.e., DEs in the opposite direction, reflecting participants learning, but the rate at which the DEs decayed were similar between groups as shown in Fig. 4.12.B (Wilcoxon rank-sum test, W=85, p=0.73).

Sham Analysis

Eleven (11) participants (52%) in the sham group experienced the stimulation even though no current was applied to them. Similar to experiment 1A, new learning curves for the sham group were generated by dissociating the DE of subjects who felt the simulation (***Sham-felt***; n=11) and those who did not (***Sham-no***; n=10). Fig. 4.13 shows that participants who perceived the stimulation learned the rotation

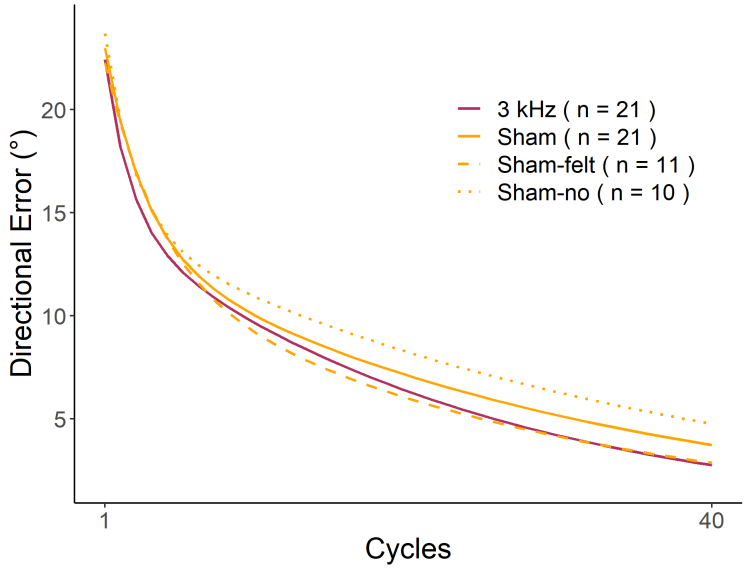

Figure 4.13: Learning curve for the sham group in experiment 1B split into participants who experience the stimulation (Sham-felt) and who did not (Sham-no).

to a rate similar to the 3 kHz group. On the other hand, subjects who did not perceive the stimulation adapted slower compared to sham, showing worst visuomotor performance.

Post Study Survey

The post-study questionnaire was responded by 41 out of 42 subjects. Both groups reported high attention level during the task performance (3 kHz: 7.7/10 ±1.9, Sham: 7.7/10 ±2.3). No correlations between the attention level and the learning rates, and the current received were found.

Also, most of the participants rated their comfort level during the experiment with an average of 8.4/10 ±1.9. However, when asked for some episodes of discomfort or pain, 37% (15 out of 41) reported to have experienced some level of discomfort. From this subgroup, 7 participants belonged to the 3 kHz group and reported an average discomfort level of 3.8/10 ±2.5. On the other hand, 8 subjects assigned to the sham group reported an average discomfort level of 4.5/10 ±2.3. Also, approxi-

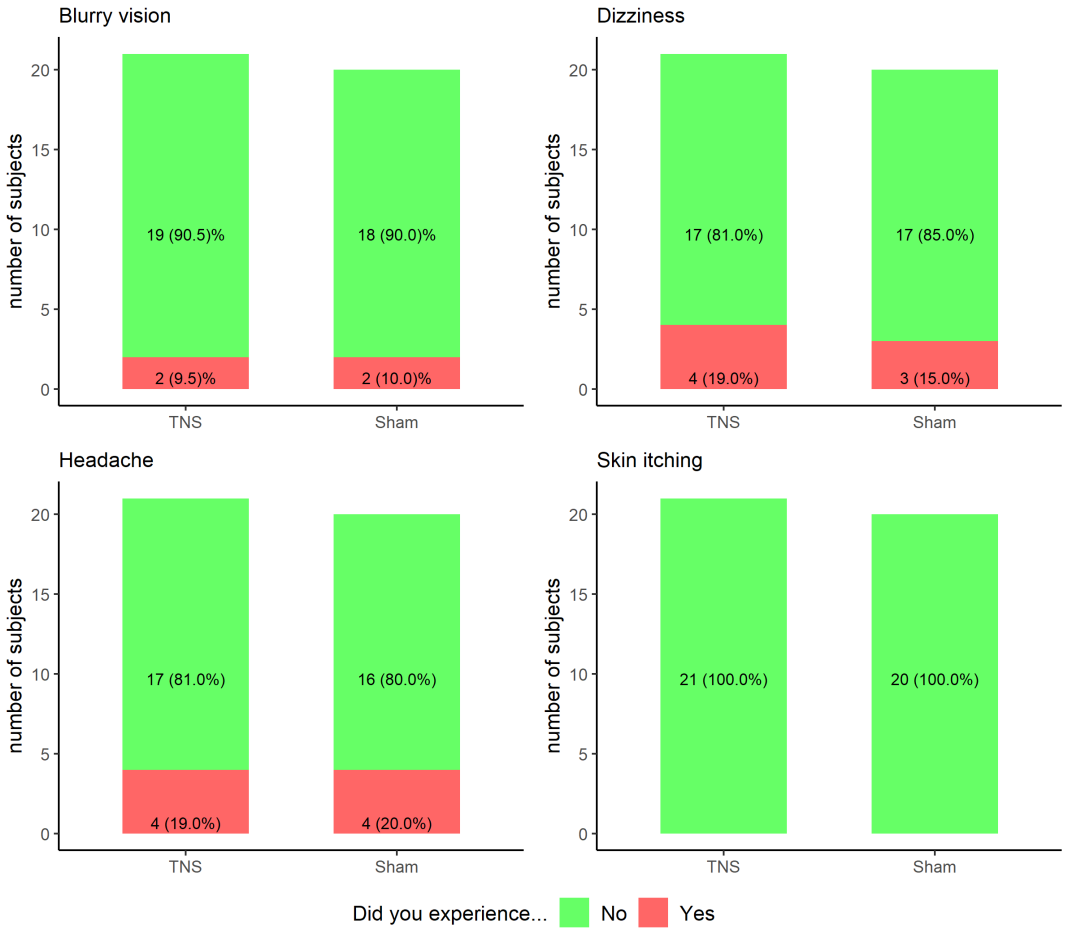

Figure 4.14: Subjects were consulted for blurry vision, dizziness, headache, and skin itching at the electrode position during or after the experiments.

mately 20% of participants or less from both groups experienced a headache (3 kHz: n=3, rate=5.3/10 ±2.2; Sham: n=3, rate=5.3/10 ±3.6) and dizziness (3 kHz: n=4, rate=2.8/10 ±2.2; Sham: n=3, rate=6/10 ±0) and less than 10% experienced blurry vision (3 kHz: n=2, rate=1.5/10 ±0.7; Sham: n=2, rate=5/10 ±4.2) during or after the experiment. Skin itching and other adverse events were not reported. Fig. 4.14 summarizes these findings.

Additionally, subjects who belonged to the active group reported tingling and pressure in the forehead during the stimulation.

4.8 Discussion

These two pilot studies provided preliminary evidence of the effects of single-session offline TNS on visuomotor adaptation. We examined clinically tested frequencies and an experimental protocol in the kilohertz range aimed to characterize which provides the best option to enhance motor learning.

Effects on Learning and Motor Performance

In experiment 1A, we assessed 60 and 120 Hz TNS, expecting those frequencies would have enhanced the learning rates during the adaptation block. However, that was not the case. Analysis of the learning curves showed that TNS at 60 Hz slowed the learning, which was more pronounced in the early adaptation phase. Both fast and slow coefficients were smaller and statistically significant compared to the 120 Hz and sham groups, which might suggest that TNS at 60 Hz had a detrimental effect on explicit and implicit mechanisms. On the other hand, the 120 Hz group ended up learning the perturbation slightly faster than the sham. However, changes in directional error were relatively modest and were not statistically significant.

In experiment 1B, we assessed TNS applied at 3 kHz. Examination of the learning curves during the rotation block suggested that the 3 kHz group learned the rotation faster than the sham group. This way, learning rate comparison suggests that 3 kHz might also affect both learning systems. Although only the slow rates were higher and statistically different from sham, the fast rates tended in the same direction ($p=0.066$). Further analysis using a permutation test showed that fast rates were also different from sham.

In addition, the 3 kHz group showed enhanced RT and PV post-stimulation. During the rotation and washout blocks, the 3 kHz group exhibited a statistically

significant increase in PV between early and late adaptation, as well as between early and late deadaptation. Similarly, during the rotation block, the 3 kHz group displayed a reduction in RT between early and late deadaptation. Thus, the 3 kHz group not only showed faster learning rates but also a more substantial reduction in RT and an increase in PV compared with the sham group, suggesting that using 3 kHz may be a superior option compared to the 120 and 60 Hz protocols for future experiments the motor domain.

In both experiments, analysis of participants' directional errors and reaction time revealed that the stimulation did not affect visuomotor performance during the baseline blocks, which involved only veridical visual feedback. On the other hand, we observed in both experiments an increase in the peak velocity after the stimulation/sham session for all groups. The increase in peak velocity in baseline 2 may be attributed to practice. Participants moved faster as they got used to the task and felt more comfortable with the 3D environment. Although having a post-stimulation baseline was a necessary control to determine whether TNS affects motor performance in unperturbed conditions [83], it might have risked some neuromodulatory effects during those approximately 5 minutes of baseline trials, which could have been used during the early phase of the rotation block. Besides this, we observed effects using 60 Hz and 3 kHz stimulation that altered the rate of learning.

Although the mechanisms behind these effects remain unknown, the Aston-Jones model may provide a possible explanation. This model relates task performance with Locus Coeruleus (LC) activity, similar to the Yerkes-Dodson law that relates performance with arousal using an inverted U shape [84]. Assuming that both frequencies were able to alter LC activity, 60 Hz TNS may disrupt the balance of the tonic firing, resulting in very low or high levels of activity at the LC, which has been linked to poor performance. On the other hand, the 3 kHz protocol may be more likely

to induce moderate tonic firing and facilitate phasic firing, which is associated with optimal performance. LC sends projections to several areas of the cortex, including the Prefrontal Cortex (PFC), associated with explicit learning, and the cerebellum with implicit learning. The ability of these stimulation protocols to alter LC activity during a visuomotor rotation task might activate these cortical areas, affecting both learning mechanisms. This perspective offers a potential explanation for the observed effects. However, further investigation needs to be done.

Sham Groups Might be Affected by Placebo Effects

In both studies, some participants in the sham group (24% in Exp. 1A and 52% in 1B) perceived the stimulation even though no current was applied. Interestingly, examining the directional error throughout the adaptation block of this sham subgroup, we observed they learned the rotation at a faster pace, similar to the best-performers groups (120 Hz and 3 kHz), suggesting a potential placebo effect. Previous studies in the brain stimulation field have reported strong placebo effects [85], which have been attributed to various factors such as the use of bulky equipment, buzzing, and noise produced by the equipment, electrode placement, among others. In our protocol, subjects in the sham group used the same electrode placement, the stimulator made the same clicking sound every time it was supposed to be active, and the experimenter mimicked the same protocol used for the active groups to look for the submaximal tolerable threshold. All these factors may have contributed to a placebo effect in part of the sham group. On the other hand, participants who did not perceive the stimulation presented the slowest learning among groups. The expectation of receiving a treatment, i.e., stimulation or a drug, has been shown to be relevant in placebo/sham-controlled studies [86]. The fact that this subgroup of the sham did not feel the stimulation might have lowered their expectations of the stimulation,

potentially affecting their performance.

Stimulation Tolerability

10% of participants in experiment 1A and 37% in 1B reported to have experienced some discomfort during the experiments. Although TNS might have produced some discomfort, it is unlikely because participants were encouraged to select a comfortable intensity but also, they could ask to reduce or stop the stimulation at any time. Additionally, subjects in the sham group also reported discomfort, even though they did not receive any current. Thus, the discomfort is likely the result of arm fatigue due to the multiple arm movements. In Chapter 6, we will try to clarify the source of the discomfort by asking participants where the discomfort is located, if any.

During experiment 1B, one participant felt lightheaded after three minutes of TNS at 3 kHz. Unfortunately, the participant did not disclose their history of fainting episodes. Syncope and dizziness has been reported in the VNS literature [87]. Considering the similarity in the mechanisms of action between TNS and VNS, individuals who have experienced fainting or vasovagal syncope were excluded to minimize the risk of such adverse events during the experiment. Despite this event, TNS was well tolerated during the stimulation session in both experiments. Participants in the 3 kHz groups were able to tolerate higher currents than the 120 and 60 Hz groups, which was in line with other studies where frequencies in the kilohertz range were used [57, 7]. Additionally, men tended to choose higher intensities when stimulation was delivered at frequencies of 60 and 120 Hz. This was not observed in experiment 1B, however, gender-wise, the sample was unbalanced with only 4 females compared to 15 males in the 3 kHz group, which makes it difficult to draw conclusions regarding a gender-intensity relationship.

Although the majority of participants did not report any serious adverse events

when specifically consulted for headaches, dizziness, blurry vision, and skin itching at the electrode site, a small percentage reported having experienced them. Although headaches and skin itching have been associated with TNS in other studies [23, 27], in these experiments is difficult to associate them exclusively as a side effect of the stimulation because subjects in the sham group also experienced them, even though no current was applied. Other factors such as the experiment duration (2 h) and the fact that the paradigm was performed in a semi-immerse VR environment might have contributed to these adverse events. For example, it is known that VR may produce cybersickness which considers dizziness, blurry vision, and headache as part of the symptoms [88]. This way, the VR environment might have contributed to the appearance of these adverse events.

4.9 Conclusion

Results from both experiments suggest that TNS may exhibit stimulation-dependent effects on visuomotor adaptation, which provides new information on the potential use of TNS in motor applications. Specifically, when TNS was delivered offline for 20 minutes at 60 Hz and 3 kHz, it was found to have the capability of altering the learning rate of a visuomotor rotation task. Interestingly, in one instance, the learning rate was reduced, while in the other, it was enhanced. A potential explanation of these effects can be found in the Aston-Jones model that relates LC activity with task performance. 60 Hz might disrupt tonic firing in the LC which is linked to poor performance. On the contrary, 3 kHz may induce moderate tonic firing and facilitate phasic firing, which is linked to optimal performance. Determining whether these effects were influenced by the frequency or the current intensity of TNS requires further investigation, which is beyond the scope of this dissertation.

Chapter 5

EFFECTS OF ONLINE TNS ON VISUOMOTOR LEARNING

5.1 Introduction

Stimulation timing is an important factor to consider when assessing brain stimulation techniques. Numerous studies have examined the effects of transcranial or cranial nerve-based stimulation methods in different contexts, considering offline (prior to task performance) and online (during task performance) protocols. Notably, online stimulation in single-session experiments is reported to have more pronounced effects. For instance, although Transcranial Alternating Current Stimulation (tACS) applied online was shown to enhance motor cortex excitability, offline tACS did not produce the same effect [89]. In another study [90], more pronounced effects on skill acquisition were observed when using online Transcranial Direct Current Stimulation (tDCS) compared to offline. Similarly, in other experiments [91], faster learning rates were observed in an explicit-sequence learning task using online anodal tDCS, compared to offline. Although no direct comparison between online and offline has been conducted for cranial nerves-based methods, several studies have shown positive using the online approach. For instance, online Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) has been shown to enhance performance in emotion recognition [92] and motivation tasks [93]. Thus, exploring the effects of online stimulation could provide valuable insights into the comprehensive understanding of the stimulation parameter space.

In the previous chapter, we observed that 60 Hz Trigeminal Nerve Stimulation (TNS) had a detrimental effect on learning, while 120 Hz stimulation tended to result

in slightly faster learning than sham. In an effort to potentially improve upon previous outcomes and provide a complete characterization of the timing effects of TNS, this chapter presents the results of a study assessing TNS using clinically tested frequencies (120 and 60 Hz) delivered online during performance of the same visuomotor learning task. We hypothesized that online stimulation would result in beneficial effects on learning. We also hypothesized that online stimulation would be more beneficial than offline stimulation. As a result, a comparison is made with the results obtained from the offline study in Chapter 4.

5.2 Participants

Sixty-five (65) subjects were recruited for this study. All subjects self-reported themselves to be right-handed and were also classified as right-handed by the Edinburgh Handedness Inventory (score $\geq$ 62.5). Two data sets were discarded: one participant complained about eye strain and abandoned the experiment, while another participant, assigned to one of the stimulation groups, was stimulated with a different type of surface electrodes that were not consistent with the ones used in this study. This way, 63 subjects (18 - 32 y/o; 23.2$\pm$3.9 y/o; 12 female and 51 male) were considered for analysis. The experimental groups were the same as experiment 1A: 120 Hz, 60 Hz, and sham.

5.3 Study Procedure

Like experiment 1A and 1B, participants begun with a familiarization block followed by a baseline block of 48 trials to assess their performance under unperturbed conditions. After the baseline block, subjects experienced an adaptation block of 200 trials involving a 30° CCW rotation of hand visual feedback and concurrent TNS or sham. Once this block was completed, subjects were asked if they felt something and,

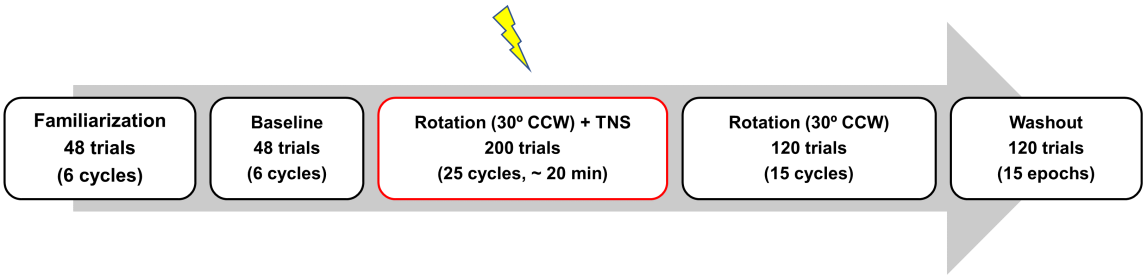

Figure 5.1: Study design for experiment 2. TNS was applied online.

if so, to sketch on a diagram of the face anatomy where they felt experienced sensations. Then, participants performed another 120 trials with rotated feedback but no stimulation. The experiment ended with a washout block of 120 trials, where the rotation was removed, and no stimulation was applied. Fig. 5.1 shows the experimental design.

5.4 Stimulation Protocol

The same stimulation protocol described in 3.5 was used. The only difference were that TNS was delivered online, concurrently with the task and the stimulation stopped when 200 trials (25 cycles) of the rotation block were completed. Based on pilot data, we estimated that participants could complete the 200 trials in approximately 20 minutes. This way, the dose duration was comparable to that of experiment 1A.

5.5 Data Analysis

5.5.1 Analyses of Baseline Performance

A one-way ANOVA was conducted to compare Directional Error (DE), Reaction Time (RT), and Peak Velocity (PV) between groups during the baseline block. Prior to the analysis, Shapiro-Wilk test confirmed that the distribution of the data was not different from normal distribution ($p > 0.05$), thus satisfying the normality require-

ments for the ANOVA test.

5.5.2 Analyses of Adaptation and Post-Adaptation Performance

DE, RT, and PV during the rotation and washout block were analyzed based on the methods described in 3.7.

Similar to experiment 1A, Kruskal-Wallis test was used to compare learning and forgetting rates because they were not normally distributed (Shapiro-Wilk test $p>0.05$). Learning curves and rates from experiment 1A, where TNS was applied offline, were compared with the results of this study. To this end, Wilcoxon test was used.

5.6 Results

The stimulation groups self-selected similar electrical currents (60 Hz: 3.3±0.8 mA; 120 Hz: 3.1±0.8 mA). A t-test showed that there was no statistically significant difference between these currents (t=-0.67, df=40, p=0.51). The duration of the stimulation session was approximately 20 minutes (120 Hz: 21±4.2 min, 60 Hz: 22±4.1 min, sham: 21.8±3 min), similar to the offline protocol used in experiment 1A. Additionally, there was no statistically significant differences (T-test, t=-0.35, df=16.45, p=0.73) in the intensity level tolerated by men (n=34, 3.2±0.8 mA) and women (n=8, 3.1±0.5 mA).

On average, participants reported in the post-study survey to have been attentive during the task performance (120 Hz: 8.1/10 ±1.8, 60 Hz: 8.5/10 ±1.3, sham: 8.2/10 ±1.8). When assessing potential correlations between the attention level, the current applied, and the learning rates, no relationships were found.

Reaction time

Fig.5.2 shows the reaction time during the baseline block. The specific reaction times per group were as follows: 120 Hz (328.3±34.6 ms), 60 Hz (348.8±66.0 ms), and sham (331.4±31.5 ms). A one-way ANOVA revealed no statistically significant differences among groups (F=1.174, p=0.316).

In the early phase of the adaptation, the reaction time reached the following mean values per group: 120 Hz (327.7±28.0 ms), 60 Hz (342.9±51.7 ms), and sham (333.0±30.0 ms). In the late phase, RT remained stable except for the sham group where we observed a reduction in RT: 120 Hz (327.9±28.6 ms), 60 Hz (337.9±56.9 ms), and sham (319.8±27.8 ms). The two-factor mixed ANOVA showed no statistically significant main effect of groups on RT (F=0.86, p=0.427). However, it showed statistically significant main effect of early/late adaptation on RT (F=6.62, p=0.013). A post hoc test revealed a statistically significant difference between early and late adaptation for sham (p<0.001), but no 120 Hz (p=0.959) nor 60 Hz (p=0.268). Also, this analysis showed that there was not a interaction between groups and early/late phase adaptation (F=2.81, p=0.068).

During the washout block, in the early phase, the reaction time per group was as follows: 120 Hz (330.7±24.4 ms), 60 Hz (338.7±61.9 ms), and sham (320.9±28.2 ms). During the late phase, RT remained approximately constant: 120 Hz (330.3±29.1 ms), 60 Hz (336.5±58.1 ms), and sham (317.8±30.6 ms). A two-factor mixed ANOVA showed no statistically significant main effects of groups (F=1.07, p=0.349) or early/late deadaptation (F=0.86, p=0.357). In addition, this analysis showed no interaction between group and early/late deadaptation (F=0.141, p=0.868).

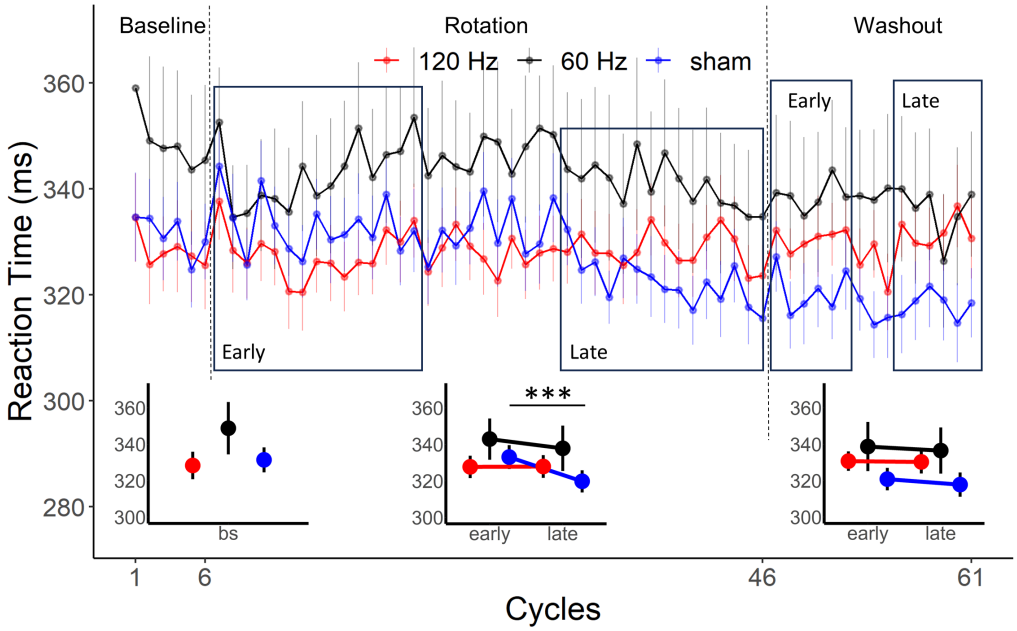

Figure 5.2: Mean reaction time (±SEM) for the 120 Hz (red), 60 Hz (black) and sham (blue) groups across experiment 2.

Peak velocities

Fig.5.3 shows the peak velocities per group during the experiment. During baseline, peak velocities were approximately 0.5 m/s (120 Hz: 0.51±0.086 m/s, 60 Hz: 0.47±0.11 m/s, sham: 0.54±0.11 m/s). A one-way ANOVA showed no statistically significant among groups (F=2.717, p=0.074).

During the rotation block, peak velocities in the early phase were distributed as follows: 120 Hz (0.49±0.07 m/s), 60 Hz (0.47±0.09 m/s), and sham (0.53±0.12 m/s). In the late phase, all groups experienced an increase in PV: 120 Hz (0.58±0.16 m/s), 60 Hz (0.56±0.14 m/s), and sham (0.59±0.15 m/s). A two-factor mixed ANOVA showed no statistically significant main effect of groups on PV (F=0.77, p=0.465). There was a statistically significant main effect of early/late adaptation on PV (F=34.04, p<0.001). A post hoc test revealed that there was a statistically significant between early and late phase of adaptation for all groups (120 Hz: p=0.001, 60 Hz: p=0.002,

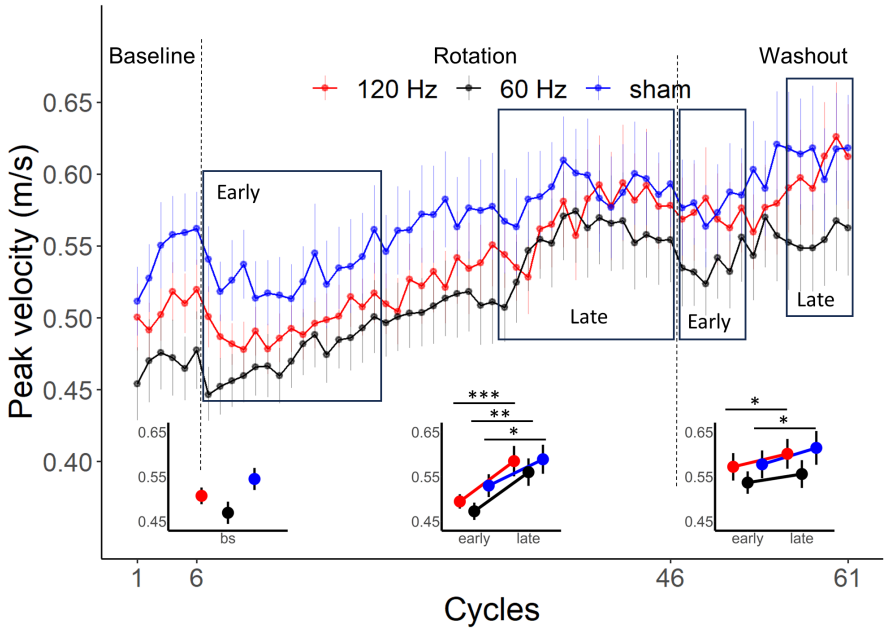

Figure 5.3: Mean peak velocity (±SEM) for the 120 Hz (red), 60 Hz (black) and sham (blue) groups across experiment 2.

sham: p=0.017). Finally, the analysis showed that there was not a statistically significant interaction between groups and early/late adaptation (F=0.548, p=0.581).

Lastly, for the washout block, the specific PV per group was the following during the early phase: 120 Hz (0.57±0.14 m/s), 60 Hz (0.54±0.11 m/s), and sham (0.58±0.14 m/s). In the late phase, PV per group slightly increased: 120 Hz (0.60±0.15 m/s), 60 Hz (0.56±0.14 m/s), and sham (0.61±0.17 m/s). A two-factor mixed ANOVA showed no statistically significant main effect of groups on PV (F=0.73, p=0.488). In contrast, there was a statistically significant main effect of early/late phase deadaptation on PV F=13.24, p<0.001). A post hoc comparison revealed a statistically significant difference between early and late adaptation for 120 Hz (p=0.048) and sham (p=0.023) but no 60 Hz (p=0.111). Finally, the analysis showed no statistically significant interaction between groups and early/late deadaptation (F=0.43, p=0.65).

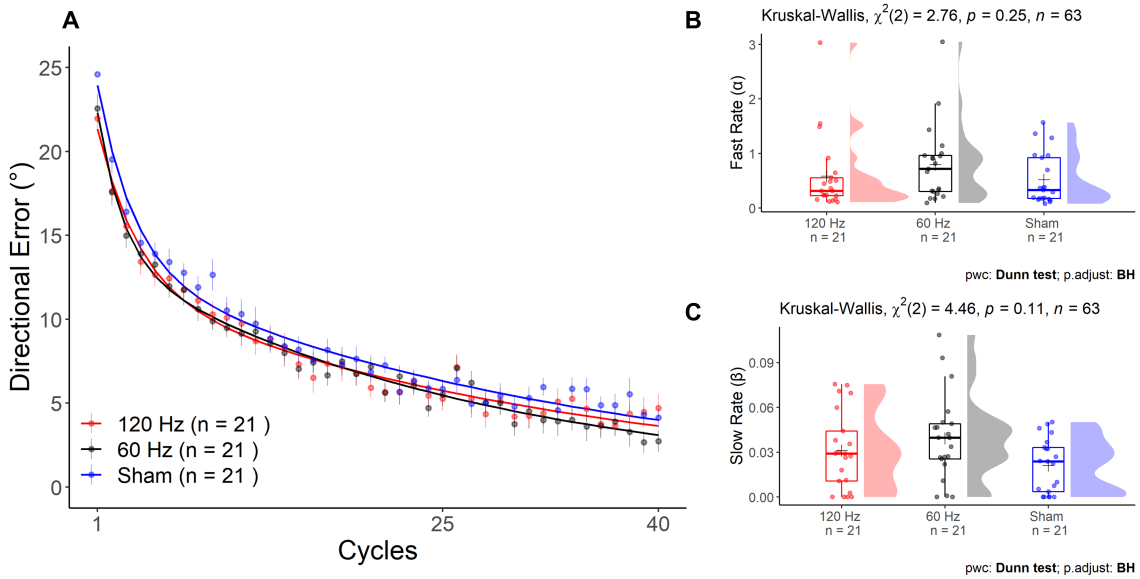

Figure 5.4: A) Learning curves. B) Fast and C) Slow rates analysis for the online study.

Directional Error

All groups showed an average directional error close to zero during the baseline block: 120 Hz (-0.25°±1.1), 60 Hz (0.48°±1.57) and Sham (0.09°±1). A one-way ANOVA showed no statistically significant differences among groups (F=1.78, p=0.177).

During the rotation block, learning curves in Fig.5.4.A shows that stimulation groups learned slightly faster than sham. However, as shown in Fig.5.4.B and C, no statistically significant differences were found among groups for fast (Kruskal-Wallis test; χ^2=2.76, df=2, p=0.25) and slow rates (Kruskal-Wallis test; χ^2=4.46, df=2, p=0.11).

For the washout block, Fig. 5.5.A shows that DEs followed the characteristic error pattern in the opposite direction but no difference in the rate at which performance returned to baseline levels between groups was found (Kruskal-Wallis test; χ^2=2.86, df=2, p=0.24).

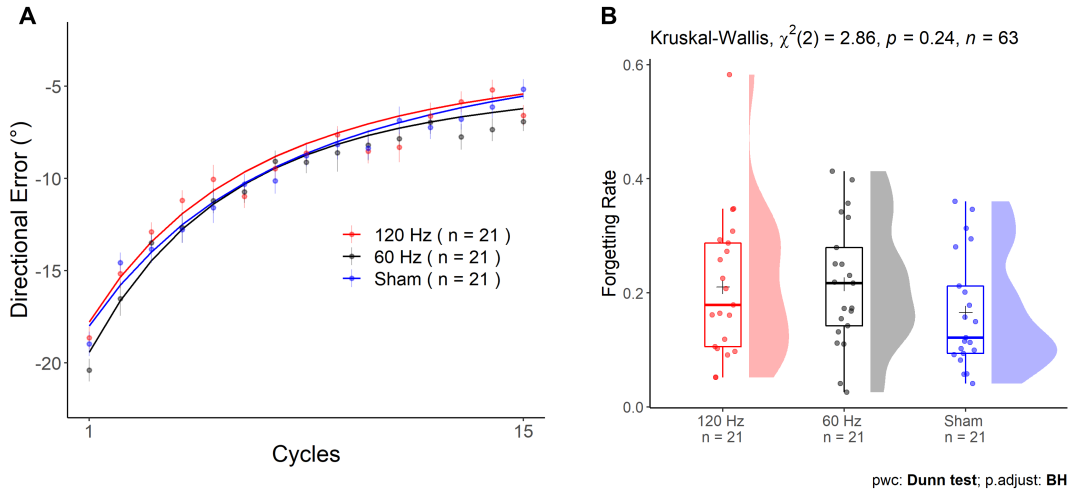

Figure 5.5: A) Single exponential model fitted to the DE washout data. B) Rate analysis.

Post Study Survey

Out of sixty-one (61) subjects who completed the post-study survey, 14 individuals (23%) experienced discomfort or pain during the experiment. On average, they rated the discomfort at 3.4/10 ±1.4, indicating a mild level. Distributed by groups, two subjects in the 120 Hz group reported discomfort (rate=2.5/10 ±0.7), five in the 60 Hz group (rate=3.4/10 ±2.6), and seven in the sham group (rate=3.6/10 ±1).

Additionally, consulted for headaches, dizziness, blurry vision, and skin itching, a small percentage of participants across all the groups reported having experienced at least one of them. For instance, only one participant in each active group reported blurred vision (120 Hz: n=1, rate=1/10; 60 Hz: n=1, rate=5/10). On the other hand, the 60 Hz group experienced 5 events (23.8%, rate=4.6/10 ±1.8) of headaches, followed by the sham group with 3 cases (14.3 %, rate=5.3/10 ±3.5) and only one case in the 120 Hz group (5%, rate=2/10). Dizziness was reported in less than 10% of each group (120 Hz: n=1, rate=7/10; 60 Hz: n=2, rate=3/10 ±0; sham: n=1, rate=6/10), similar to skin itching at the electrode site (120 Hz: n=1, rate=3/10; 60

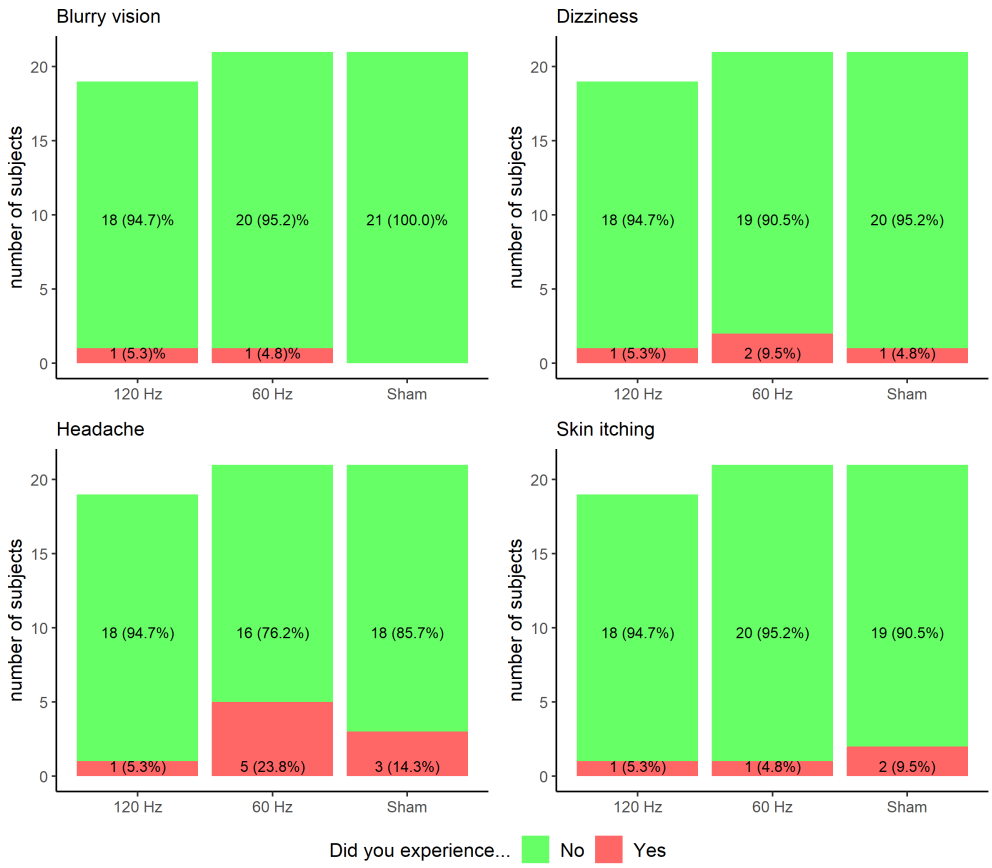

Figure 5.6: Post-study responses sorted by group when participants were consulted for headaches, blurry vision, dizziness and skin itching.

Hz: n=1, rate=4/10; sham: n=2, rate=5/10 ±4.2). Fig. 5.6 summarizes the side effects reported.

Sham Analysis

Similar to experiments 1A and 1B, 8 out of 21 subjects (38%) from the sham group reported to have perceived the stimulation even though no current was applied to them. Fig.5.7 illustrates the learning curves for the sham group, divided into those who perceived TNS (***Sham-felt***; n=8) and those did not (***Sham-no***; n=13), as well as the active groups. It is possible to observe that the subgroup ***Sham-felt*** (blue dashed line) demonstrated a similar learning rate compared to the active groups,

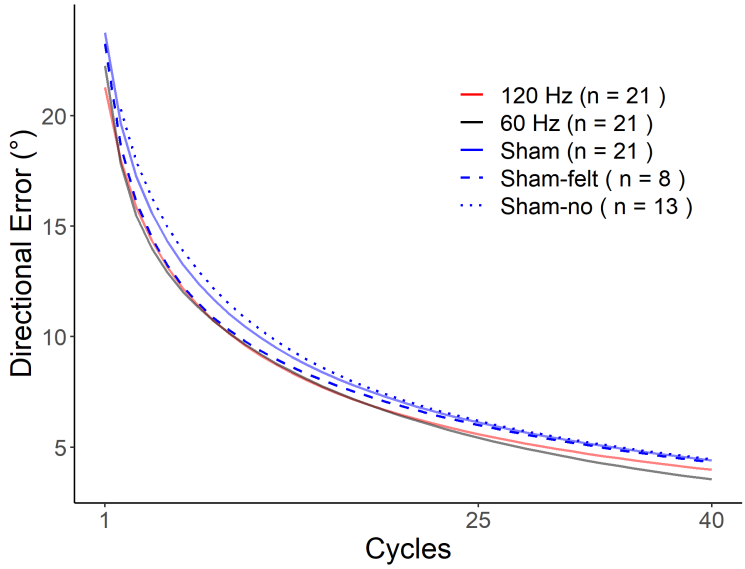

Figure 5.7: Sham directional error was split into subjects who felt (Sham-felt) the stimulation and who did not (Sham-no). Learning curves for the active groups and the subgroups of the sham are displayed (Sham-no: blue-dotted line, Sham-felt: dashed-line)

during the initial phase of learning. Then, it diverged tending to the sham overall performance. In contrast, the subgroup ***Sham-no*** (dotted blue-line) exhibited the slowest learning in the early phase of the adaptation but eventually converged to sham levels by the end of the rotation block.

Comparison between offline vs online 120 Hz and 60 Hz studies

Fig. 5.8.A shows the learning curves obtained from two different cohorts of subjects who received TNS at 120 Hz, applied online (maroon-bold line) and offline (red-bold line). As suggested by the overlapped learning curves, learning rates (Fig. 5.8.B and C) with online 120 Hz were similar to those previously observed with offline 120 Hz TNS (Wilcoxon test, α: W=246, p=0.53; β: W=231, p=0.8). On the other hand, learning curves showed in Fig. 5.9.A suggests that 60 Hz online was markedly faster than those observed previously with 60 Hz-offline. This observation was confirmed by comparing the learning rates (Wilcoxon test, α: W=71, p <0.001; β: W=101,

p=0.0027).

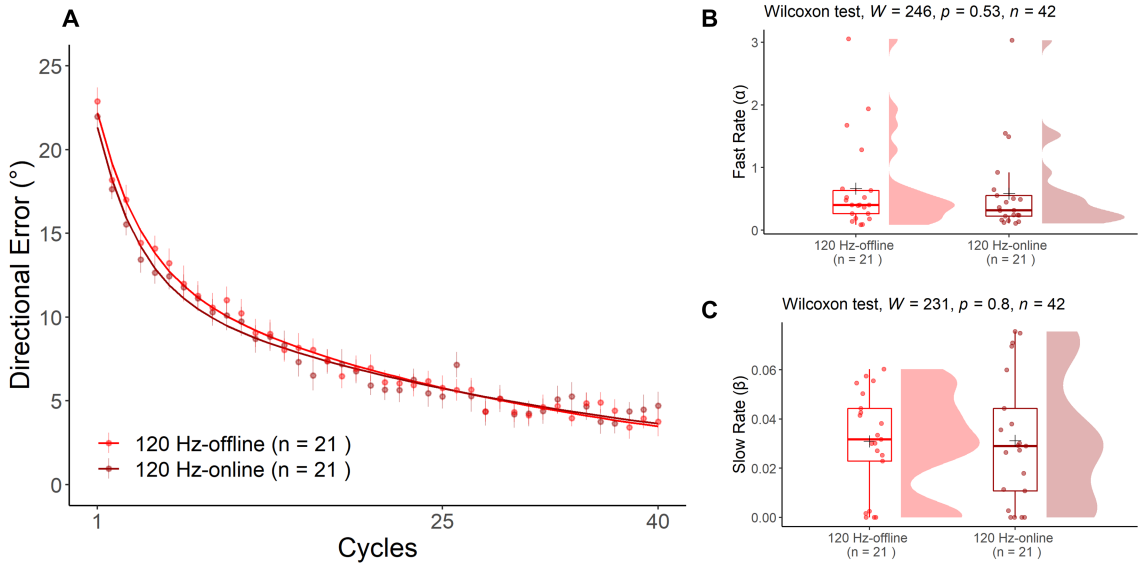

Figure 5.8: A) Learning curves fitted to the DE for the 120 Hz group applied online (maroon bold line) and offline (red bold line). B) Fast and C) slow rates comparison between 120 Hz applied offline and online.

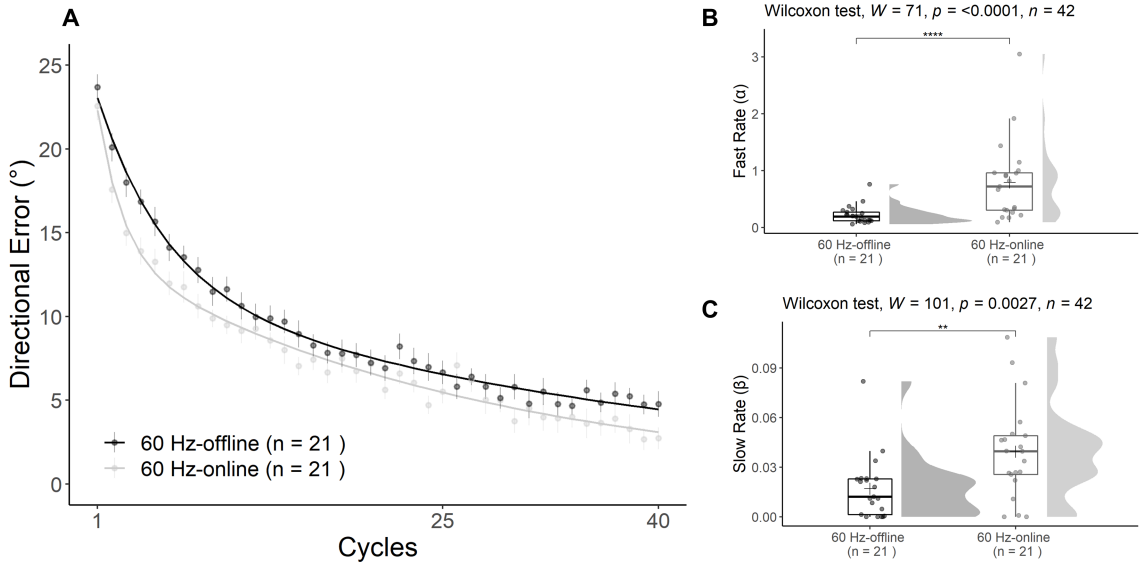

Figure 5.9: A) Learning curves fitted to the DE for the 60 Hz group applied online (gray bold line) and offline (black bold line). B) Fast and C) slow rates comparison between 60 Hz applied offline and online.

Effects on Learning Rates and Comparison with Offline TNS

This study explored the effects of 120 and 60 Hz TNS protocols delivered online on visuomotor learning. Given the results obtained in experiment 1A, which did not show learning improvement, we assessed the same 120 and 60 Hz protocols, but applied TNS during task performance (online). We expected to find more pronounced effects of TNS, given the encouraging results observed using online stimulation in tACS, tDCS, and taVNS. However, even though learning curves from active groups showed slightly faster adaptation under perturbed conditions, learning rates analysis showed no statistically significant differences compared to sham.

Interestingly, when results from this experiment were compared directly with those observed in experiment 1A (offline TNS), significantly faster learning rates were observed for the online 60 Hz protocol. This result suggests that TNS-dependent effects on visuomotor adaptation depend on the timing of stimulation relative to task performance, at least for 60 Hz stimulation. In contrast, the online 120 Hz group maintained a similar learning rate as when this stimulation frequency was applied offline, showing no differences in learning between studies.

The online effects of transcranial stimulation have been attributed to the modulation of cortical excitability through the alteration of membrane properties by weak electric currents [94]. On the other hand, TNS effects are thought to be produced by the activation of Locus Coeruleus (LC), subsequent release of Norepinephrine (NE) across the brain, and resulting modulation of synaptic plasticity. Because neurotransmitter release is a slow process, this mechanism might be more suitable for offline stimulation rather than online. However, the timing effects observed suggest another mechanism of action. Simulation studies have suggested that supraorbital

TNS might also generate transcranial effects [22], activating frontal areas. Explicit learning has been associated with the activation of the frontal cortex, particularly with the PFC, a brain region involved in high-level cognition, decision-making and strategic learning. As depicted in Fig.5.9, online TNS at 60 Hz showed relatively rapid learning compared to offline TNS , an effect that was more pronounced in the early adaptation phase. This suggests active involvement of explicit learning mechanisms, which are thought to be mediated by frontal areas.

Sham Groups Performance

In this study, 38% of the sham group participants experienced sensations consistent with stimulation, similar to experiments 1A and 1B. It is worth noting that no electrical current was applied in any of these studies, despite the electrodes being placed on the forehead as in the active groups. Consistent with the observations in experiments 1A and 1B, individuals who reported feeling TNS initially showed rapid learning at a similar rate as active groups (blue dashed line, sham-felt). However, after approximately 15 cycles DEs deviated slightly, converging towards the levels in the sham-no group, which exhibited the slowest learning throughout the rotation block. This observation suggests that part of the sham might have been influenced by a placebo effect which could be masking stronger TNS effects.

Stimulation tolerability

In line with findings in experiments 1A and 1B, 23% of all participants experienced some level of discomfort. Interestingly, the sham group reported the highest number of discomfort cases (7 cases). This observation suggests that the discomfort experienced may not be primarily caused by TNS. Instead, it likely comes from arm fatigue related to the repetitive nature of the reaching task. This issue will be addressed in the next

Chapter.

Furthermore, participants were inquired specifically about headaches, blurry vision, skin itching, and dizziness. Headaches were reported by the highest number of participants with 5 (23.8%) in the 60 Hz group, followed by 3 (14.3 %) in the sham group. These findings align with the results of experiment 1A, where 3 (14.3%) cases were reported in the 60 Hz group and 2 (10.5%) cases in the sham. In both cases, 120 Hz reported fewer than 10% of headache cases. While headaches have been associated with potential side effects in a small percentage of cases, it is noteworthy they were also reported in the sham group. This suggests that some of these headaches may be related to the experimental conditions rather than TNS itself, as discussed in Chapter 4. Factors such as the duration of the experiment, and prolonged screen exposure in VR settings can contribute to cybersickness which manifests as headaches and eyestrain, among other side effects. In fact, one participant discontinued the experiment due to eyestrain.

5.8 Conclusion

This study aimed to evaluate the effects of online TNS using clinically used frequencies on visuomotor learning. Although we observed a slight improvement in learning for the active groups, no statistically significant differences were found compared to sham.

Interestingly, in comparison to the findings from the offline study (Experiment 1A), the 60 Frequency, which previously resulted in slow learning, demonstrated improved performance when delivered online. TNS effects are associated to the release of NE to the cortex by the LC, which could be a slow process, more suitable for offline stimulation. The unexpected outcome with online stimulation might suggests an alternative mechanism to exert its effects, related to transcranial effects and the

ability to generate cortical excitability like tDCs. However, further investigation is required to fully understand and explore this possibility.

Although the 60 Hz protocol has shown the ability to modulate learning behavior and consequently, generate plasticity-related changes, it did not significantly accelerate learning compared to the sham group. As a result, the next chapter will focus on further investigating motor learning enhancement using a 3 kHz offline protocol, which showed the most promising results thus far.

Chapter 6

EFFECTS OF TNS AND ONS DELIVERED INDEPENDENTLY AND CONCURRENTLY ON VISUOMOTOR LEARNING.

6.1 Introduction

Recently, several investigators have attempted to combine neuromodulation modalities in an effort to achieve a stronger behavioral and therapeutic effects. For instance, combined Transcranial Direct Current Stimulation (tDCS) and Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) applied during performance of a working memory task showed a tendency for larger improvements than expected based on results using these modalities individually, suggesting a potential synergistic effect [47]. Similarly, another recent study that combined Trigeminal Nerve Stimulation (TNS) with Occipital Nerve Stimulation (ONS) showed strong therapeutic effects on migraine [6].

Thus, a valid and potentially valuable approach involves combining TNS with another modality to enhance the beneficial effects observed using the 3 kHz offline TNS protocol. ONS presents itself as a promising candidate for such joint stimulation, due to its convergence onto similar brainstem structures as the trigeminal nerve. More specifically, ONS has the potential to activate brain circuits responsible for endogenous neuromodulation of memory circuits via Locus Coeruleus (LC) mediated Norepinephrine (NE) release, similar to Vagus Nerve Stimulation (VNS) and TNS [46]. Supporting this notion, a recent paper reported that ONS enhanced associative memory in in older adults [5]. Furthermore, a new hypothesis suggests that the trigeminal and occipital nerve projections to the LC may play a role in the beneficial

effects attributed to tDCS [52], which has been used in various contexts to study behavior and cognition, as well as in clinical settings for psychiatric disorders. Consequently, exploring ONS individually, and in combination with TNS, holds promise in the context of efforts to enhance motor learning.

In this study, we explored the feasibility of stimulating the trigeminal and occipital nerves both independently and simultaneously in order to determine which neuromodulation approach provides the strongest effects on motor learning. To this end, we used the best performing protocol from previous experiments (1A, 1B and 2), i.e., the 3 kHz offline, and assessed the effects of each approach on a visuomotor learning task. Based on previous experiments employing dual neuromodulation approaches, we hypothesized that combining TNS and ONS would result in a synergistic effect, leading to enhanced learning compared to each modality delivered individually.

6.2 Participants

Eighty-eight (88) subjects participated in this single-blind sham-controlled study. All participants described themselves as right-handed. One subject was classified as mixed-handed according to the Edinburgh Handedness Inventory (score=50) but was also considered for the study. Four (4) subjects' data sets were discarded for the following reasons: technical issues (1), participation in a previous TNS study from our lab (1), data with more than 10% of movements removed during the data cleaning process (1), and inability to complete the experiment due to lightheadedness (1). In this last case, similar to the case reported in 4.2.2, the participant felt dizzy and lightheaded after less than 2 minutes of receiving stimulation. After his recovery, he commented that he has a fear of needles and always experienced the feeling of fainting during blood draw. This information was not disclosed during eligibility questionnaire questions and the consent form signing, even though these procedures

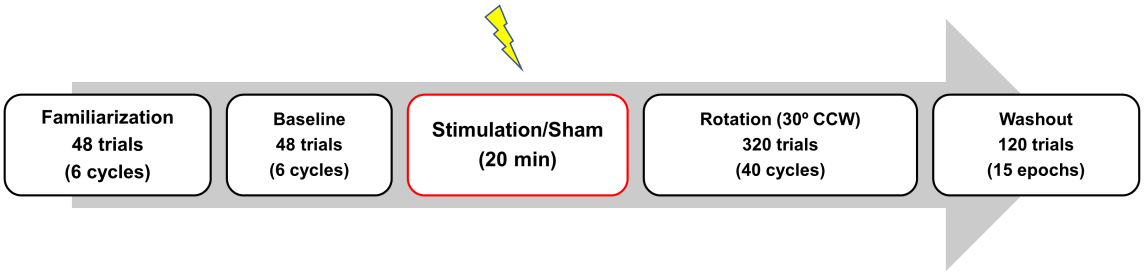

Figure 6.1: Experimental design for the ONS/TNS study.

specifically asked about history of vaso-vagal syncope and fainting episodes. He fully recovered after the stimulation stopped. The remaining eighty-four (84) subjects (18 - 34 y/o; 22.9±3.2 y/o; 27 female and 57 male) were randomly assigned one of 4 groups: TNS, ONS, TNS+ONS, and sham.

6.3 Apparatus and Behavioral Task

In this study, we used the same apparatus and behavioral task as described in Chapter 4. However, we modified the experimental design of the visuomotor rotation task by removing the post-stimulation baseline block used in previous offline TNS studies (Exp. 1A and 1B), which reduced the time between the end of the stimulation session and the beginning of the rotation block. This was done to mitigate concerns that having the subjects perform an extra 5 minutes baseline might jeopardize the window for potential neuromodulatory effects. As a result, participants experienced the rotation of their hand visual feedback immediately after receiving the stimulation, maximizing the probability that neuromodulation could influence motor adaptation. Fig. 6.1 shows the experimental design.

6.4 Stimulation Protocol

6.4.1 Active Groups Intervention

Similar to the experiments described in previous chapters, TNS was delivered by using two round 1.25 in (3.2 cm) diameter surface electrodes (Axelgaard Manufacturing Co., Ltd.) placed over the forehead, thereby targeting supraorbital branches of the trigeminal nerve. ONS was delivered using two round 1.5 in (3.81 cm) diameter carbon conductive rubber electrodes (Caputron Medical Products LLC.) combined with soaked-saline sponges. Rubber electrodes were placed 2 cm laterally from the occipital protuberance (Inion) targeting the bilateral branches of the greater occipital nerve and C2/C3 dermatomes. Each sponge was soaked with 6 ml of 0.9% sodium chloride solution (saline). Electrodes were replaced every 21 participants and sponges were used only once per subject.

Fig. 6.2 shows a schematic diagram of the TNS/ONS setup up that allowed delivery of stimulation to the forehead and the back of the head either independently or concurrently. To this end, two (2) DS8R (Digitimer Ltd.) stimulators triggered by a dual-function generator (AFG 3022B, Tektronix Ltd.) with zero delay were used. All devices were connected to a laptop and controlled by a custom GUI written in MATLAB that allowed the experimenter to deliver either TNS, ONS or TNS+ONS with the same parameters used in experiment 1B. Thus, TNS and ONS were delivered at 3 kHz, using a pulse width of 50 μs, and an interphase interval of 1 μs for 20 minutes in cycles of 30 seconds ON and 30 seconds OFF. Similar to previous stimulation protocols, the current was ramped up/down over 5 seconds to make the ON/OFF transitions more comfortable.

TNS and ONS electrodes were placed on all participants. However, depending on the group assignment, either TNS, ONS, or both (TNS+ONS) were activated during

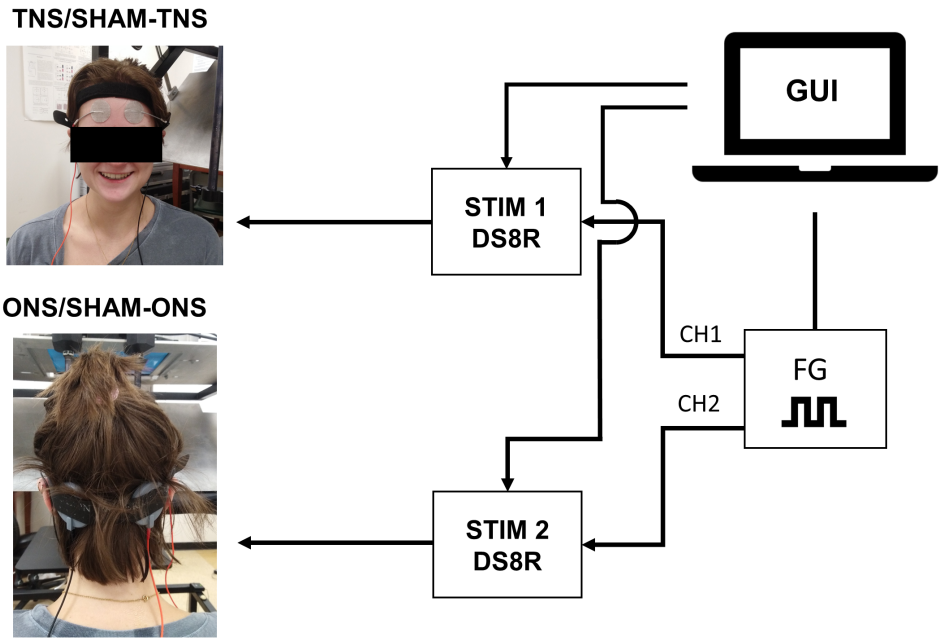

Figure 6.2: Schematic diagram of TNS/ONS setup.

the stimulation block. Despite this, all subjects, including those in the sham group, were informed the stimulation would be applied on the forehead and the back of the head concurrently using small currents, and because of that, they might or might not feel it. Also, they were told that a comfortable intensity threshold needed to be determined separately for each location before delivering concurrent stimulation. Finally, similar to previous studies, they were told that they should not feel any pain or discomfort and they could reduce the current anytime.

For concurrent stimulation (TNS+ONS group), the forehead and back of the head were stimulated with a submaximal tolerable current (see Fig. 6.3.A). This required the experimenter to perform a threshold search for each location separately. Here the current increased as long as participants were comfortable, the participant requested to stop at a particular level, or the current reached the safety threshold. The threshold search began with ONS at 2 mA and increased in 1 mA steps until the perceptual

threshold level or 7 mA was reached. Then, the increment changed to 0.1 mA, and the current increased until the submaximal tolerable threshold or 16 mA (safety threshold for ONS) was reached. After the intensity was found, ONS was turned off, and the same procedure was performed for TNS. In this case, the current went from 0 to the perceptual threshold in steps of 1 mA or 5 mA. Then, the increment also changed to 0.1 mA, and the intensity increased until the submaximal tolerable threshold or 13 mA (safety threshold for TNS) was reached. Finally, TNS and ONS were delivered concurrently using the current levels determined in the individual threshold searches. The experimenter assessed the participant's comfort, and currents were adjusted if necessary. Once the threshold search was completed, the stimulation was delivered for 20 min, and subjects were instructed to watch the same sequence of neutral images used in experiments 1A and B.

For independent stimulation (TNS and ONS groups), the individual threshold search was performed only for the active location. However, in order to follow the same protocol described above, the inactive location received sham stimulation (see subsection 6.4.2). Then, both locations were stimulated concurrently during the first 30 seconds cycle, and then only the assigned stimulation location was active for the remainder of the stimulation block (see Fig. 6.3.B and C).

Once participants completed the stimulation block, the electrodes were removed and they were asked to indicate whether they felt the stimulation. If so, they sketched where they felt it using a diagram of the face and the back of the head anatomy. Following this, they immediately performed the rotation block.

6.4.2 Sham Intervention

Unlike experiments 1A, 1B, and 2, in which the sham group did not receive stimulation, this study used an active-sham approach to reinforce subject blindness. To

achieve this, participants received 60 seconds of fixed current at each location, divided into two doses of equal duration. The first dose was administered during the threshold search, while the second dose was given during the initial 30-second cycle of the stimulation session. The currents used for sham-TNS and sham-ONS were 6 mA and 9 mA, respectively, resulting in approximately equivalent current densities (ONS: 0.789 mA/cm_2, TNS: 0.746 mA/cm_2). These intensities attempted to evoke the perception of the stimulation using the lowest current possible. Other parameters such as frequency and pulse width were the same as those in the active groups. Thus, the experimenter delivered the fixed level for 30 seconds and then pretended to increase the current approximately up to one minute after the stimulation was turned off. If the subject did not request to stop at that time, the experimenter indicated that the safety threshold was reached. This procedure was performed first for sham-ONS and then for sham-TNS. If either one or both fixed currents produced discomfort, they were reduced to a well-tolerated level. Finally, both TNS and ONS were delivered concurrently for 30 seconds and then turned off for the remainder of the stimulation block (See Fig. 6.3.D).

6.5 Post Study Survey

Following our standard protocol, upon the conclusion of the experiment, participants were requested to complete an online post-study survey. In conjunction with the inquiries outlined in 3.6, three additional questions were included to enhance the evaluation of previously identified trends discussed in earlier chapters. For instance, in experiments 1A, 1B, and 2, between 10% and 37% of participants reported experiencing pain or discomfort but it was uncertain whether the discomfort reported was produced by arm fatigue due to the multiple arm movements performed or the applied stimulation. Therefore, we added a question to determine the source of the

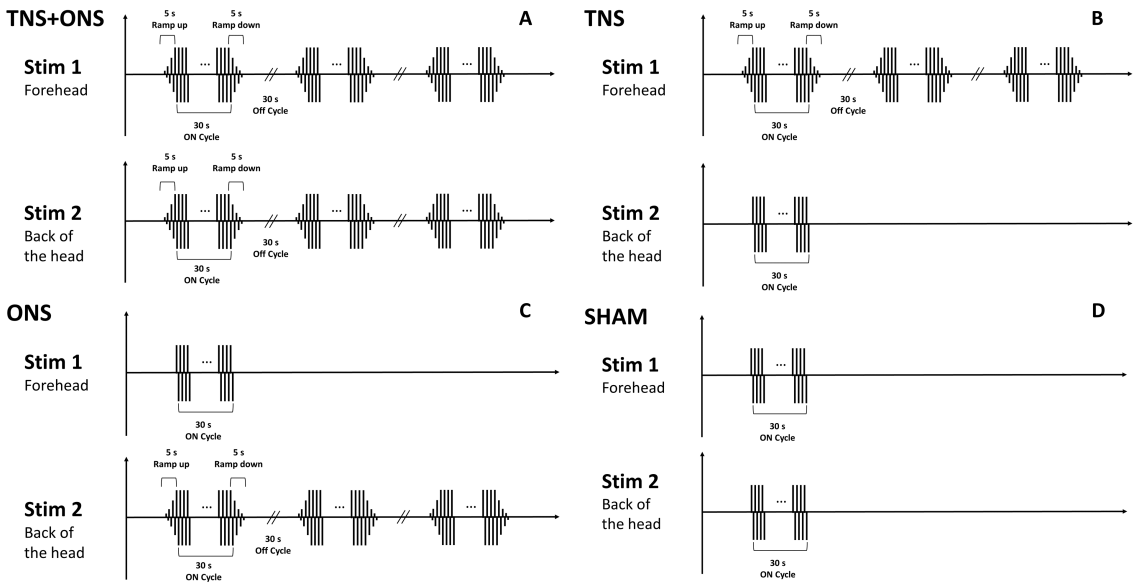

Figure 6.3: Stimulation protocol for A) TNS+ONS, B) TNS, C) ONS, and D) sham groups. Stimulation applied on the forehead and the back of the head was delivered using two DS8R stimulators (Stim 1 and 2), one for each location.

discomfort. Consequently, participants were inquired where the discomfort was localized, providing alternatives such as the forehead, back of the head, or the right arm.

Furthermore, we included a question to evaluate the effectiveness of the sham intervention. In this regard, participants were asked if they felt they had received stimulation or not and, if so, where they thought the stimulation had been applied. Additionally, to gain deeper insights into possible placebo effects, individuals who did not receive stimulation but perceived that they did, were asked if they noticed any enhancement in their motor performance during the experiments.

6.6 Data Analysis

6.6.1 Analyses of Baseline Performance

After checking normality using the Shapiro-Wilk test, Directional Error (DE), Peak Velocity (PV) and Reaction Time (RT) comparison between groups during the baseline block were tested using a one-way ANOVA.

6.6.2 Analyses of Adaptation and Post-Adaptation Performance

DE, PV, and RT during the rotation and washout block were analyzed based on the methods described in 3.7. In addition, similar to experiment 1A, 1B, and 2, Kruskal-Wallis test was used to compare learning and forgetting rates because they were not normally distributed (Shapiro-Wilk test $p>0.05$).

6.7 Results

On average, participants who received TNS and ONS independently were able to tolerate 7.2±2.6 mA and 14.0±2.8 mA, respectively. On the other hand, subjects who received TNS and ONS concurrently received 8.5±2.1 mA (for TNS) and 12.8±3.0 mA (for ONS), on average. No significant differences were found between intensities levels delivered to the same location, depending on whether the stimulation was delivered concurrently or independently (T-test, ONS: $t(39.8) = -1.38$, $p = 0.175$; TNS: $t(38.7) = 1.76$, $p = 0.086$). Fig.6.4 shows the distribution of intensities tolerated by participants per group.

Reaction Time

Fig.6.5 shows the reaction time throughout the experiment for all groups. In the baseline, a one-way ANOVA revealed that were was not statistically significant dif-

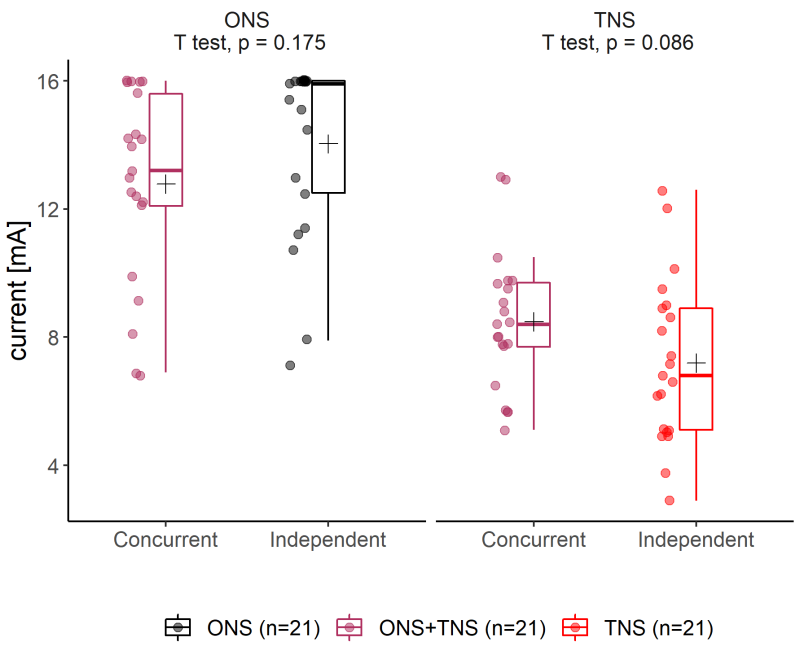

Figure 6.4: Left:Current tolerated during ONS delivered independently (ONS) and concurrently with TNS (TNS+ONS). Right: Current tolerated during TNS delivered independently (TNS) and concurrently with ONS (TNS+ONS). No significant differences were found between groups

ferences in reaction time between groups (F=1.191, p=0.319). Specifically for each group, the mean RT was as follows: TNS (335.0±43.6 ms), ONS (324.9±45.8 ms), TNS+ONS (344.8±54.4 ms), and sham (321.3±32.0 ms).

In the early phase of the adaptation, the mean RT for each group was as follows: TNS (335.7±41.0 ms), ONS (332.5±50.6 ms), TNS+ONS (341.5±53.7 ms), and sham (320.3±30.8 ms). All groups experience a slight reduction of the reaction time in the late deadaptation: TNS (325.3±38.1 ms), ONS (329.1±53.6 ms), TNS+ONS (336.0±37.0 ms), and sham (313.1±33.9 ms). A two-factor mixed ANOVA showed no statistically significant effect of groups on RT (F=0.88, p=0.456). However, it showed a statistically significant main effect of early/late phase on RT (F=17.68, $p < 0.001$). A post hoc pairwise comparison showed that there was a statistically significant difference between early and late adaptation for TNS (p=0.005), TNS+ONS (p=0.021)

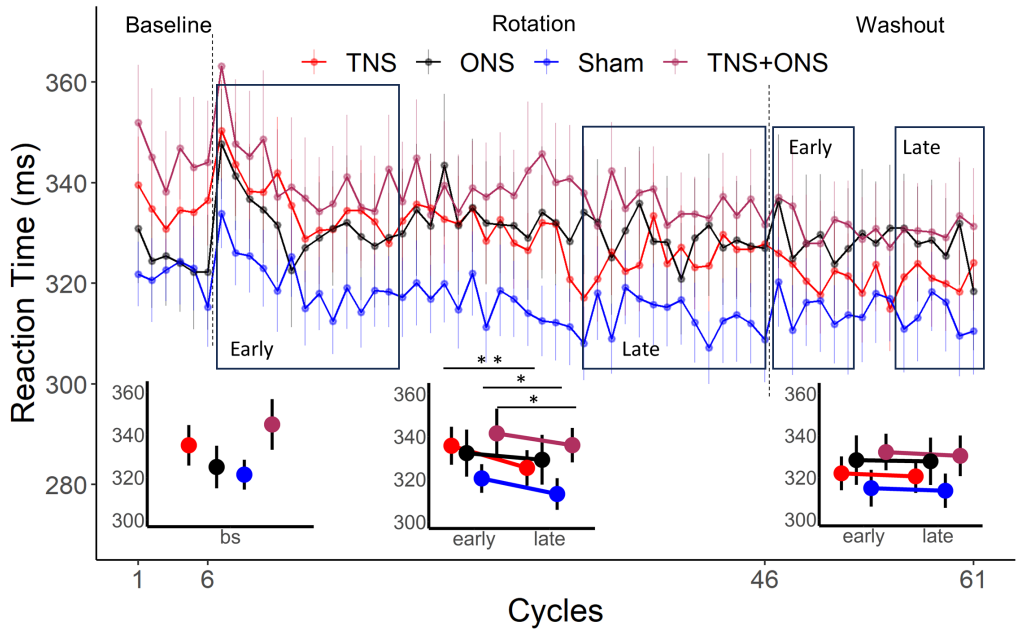

Figure 6.5: Mean reaction time (±SEM) for the TNS (red), ONS (black), TNS+ONS (maroon), and sham (blue) groups across experiment 3.

and sham (p=0.021) but not for ONS (p=0.457). Moreover, no statistically significant interaction between groups and early/late phase was found (F=0.796, p=0.5).

Finally, the reaction time reached a plateau during the washout block, the mean reaction time for the early adaptation phase for each group was as follows: TNS (322.0±37.1 ms), ONS (328.3±54.2 ms), TNS+ONS (332.1±40.0 ms), and sham (314.9±40.2 ms). In the last phase, the mean RT was the following: TNS (320.5 36.3 ms), ONS (327.7±51.9 ms), TNS+ONS (330.3±44.5 ms), and sham (313.7±37.5 ms). A two-factor mixed ANOVA showed no statistically significant main effect of groups (F=0.52, p=0.668) or (F=0.45, p=0.504) early/late phase on RT. Also, there was no interaction between groups and early/late deadaptation (F=0.014, p=0.998).

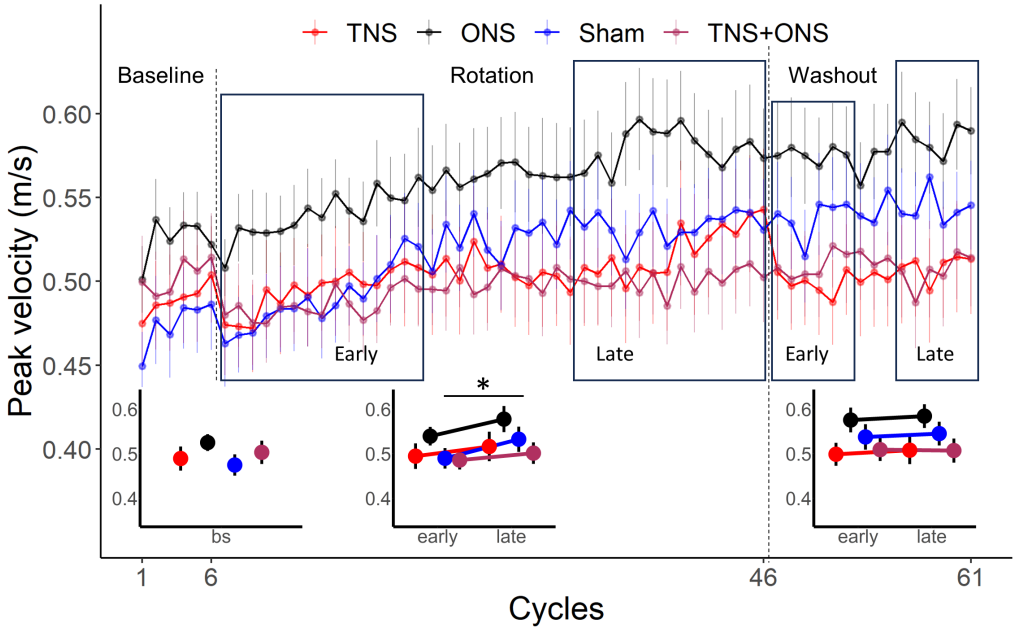

Figure 6.6: Mean Peak Velocity (±SEM) for the TNS (red), ONS (black), TNS+ONS (maroon), and sham (blue) groups across experiment 3.

Peak Velocity

Fig. 6.6 shows the peak velocity data for all groups. The figure indicates that mean PV for all groups tended to increase across the experiment. During the baseline block, average PV for each group were the following: TNS (0.49±0.12 m/s), ONS (0.53±0.09 m/s), TNS+ONS (0.50±0.12 m/s), and sham (0.47±0.11 m/s). No significant differences were found between groups (F=0.786, p=0.505).

During the rotation block, all groups saw their peak velocity slightly increase as the experiment progressed. During the early phase of the adaptation, the mean PV for each group was as follows: TNS (0.49±0.13 m/s), ONS (0.54±0.10 m/s), TNS+ONS(0.49±0.10 m/s), and sham (0.49±0.11 m/s). For the late phase, the mean PV was as follows: TNS (0.52±0.15 m/s), ONS (0.58±0.13 m/s), TNS+ONS (0.50±0.11 m/s), and sham (0.53±0.13 m/s). A two-factor mixed ANOVA showed no statistically significant main effect of groups (F=1.30, p=0.28) on PV. However,

there was a statistically significant effect of early/late phases (F=12.48, p<0.001). A post-hoc comparison revealed a statistically significant difference between early and late phases for sham (p=0.027) but not for ONS (p=0.094), TNS (p=0.07), and TNS+ONS (p=0.283). Additionally, the analysis also revealed that there was not a statistically significant interaction between groups and early/late adaptation phases (F=0.6, p=0.62).

During the washout, particularly in the early phase, the mean PV was as follows: TNS (0.50±0.12 m/s), ONS (0.58±0.13 m/s), TNS+ONS (0.51±0.12 m/s), and Sham (0.54±0.13 m/s). In the late phase, participants maintained the mean PV: TNS (0.51±0.14 m/s), ONS (0.58±0.12 m/s), TNS+ONS (0.51±0.12 m/s) and sham (0.55±0.12 m/s). A two-factor mixed ANOVA showed no statistically significant main effects of groups (F=1.75, p=0.163) or early/late phases (F=0.94, p=0.334) on PV. Also, this analysis showed no statistically significant interaction between groups and early/late deadaptation (F=0.601, p=0.62).

Directional Error

The mean directional error during baseline for all groups was -0.17 ±0.13° and the average for each group was the following: TNS (-0.09±0.97°), ONS (-0.037±1.42°), TNS+ONS (-0.053±1.3°) and sham (-0.48±1.4°). A one-way ANOVA showed no statistically significant differences among groups (F=0.575, p=0.633).

Fig. 6.7.A illustrates the directional error across the rotation block and the respective learning curves for each group. ONS exhibited the fastest learning rates. The next group with the fastest learning curve was TNS+ONS, followed by the sham group. Interestingly, the TNS group demonstrated the slowest decay in the initial deadaptation phase and converging to sham level in the late phase. This observation contrast with the results observed in experiment 1B where TNS at 3 kHz showed

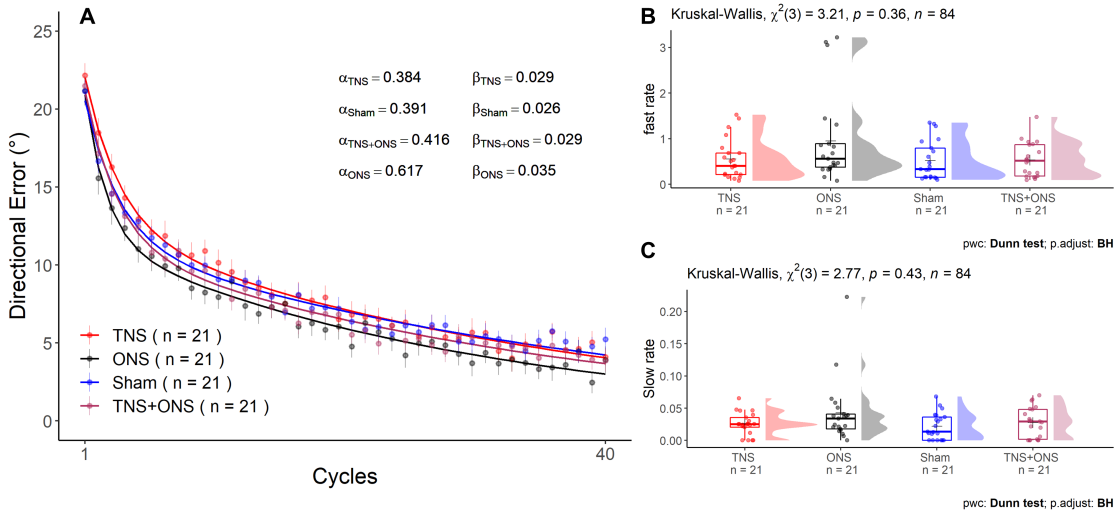

Figure 6.7: A) Learning curves. B) Fast and C) Slow rates comparison.

faster learning compared to sham. Despite the observed trends in the learning curves, the analysis of learning rates (Fig. 6.7.B and C) revealed no statistically significant difference in fast (Kruskal-Wallis test; χ^2=3.21, df=3, p=0.36) and slow learning rates (Kruskal-Wallis test; χ^2=2.77, df=3, p=0.43).

Finally, forgetting curves fitted to the DE data in the washout block are shown in Fig. 6.8. It is possible to observe that the sham group tended to return to baseline levels faster than the active groups. However, rates analysis (Fig. 6.8.B) did no reveal statistically significant differences among them (Kruskal-Wallis test; χ^2=1.22, df=3, p=0.75).

Post Study Survey

The post-study survey was responded for 81 out of 84 participants. It revealed that 17.3% (14 out of 81) of participants experienced discomfort or pain during the study, rated with 4.3±1.4 within a 1-10 scale. When consulted for the location of the discomfort, 78.6% (11 out of 14) indicated the right arm and shoulder, 14.3% (2 out of 14) the forehead, and 7.1% (1 out of 14) reported both. The three participants who

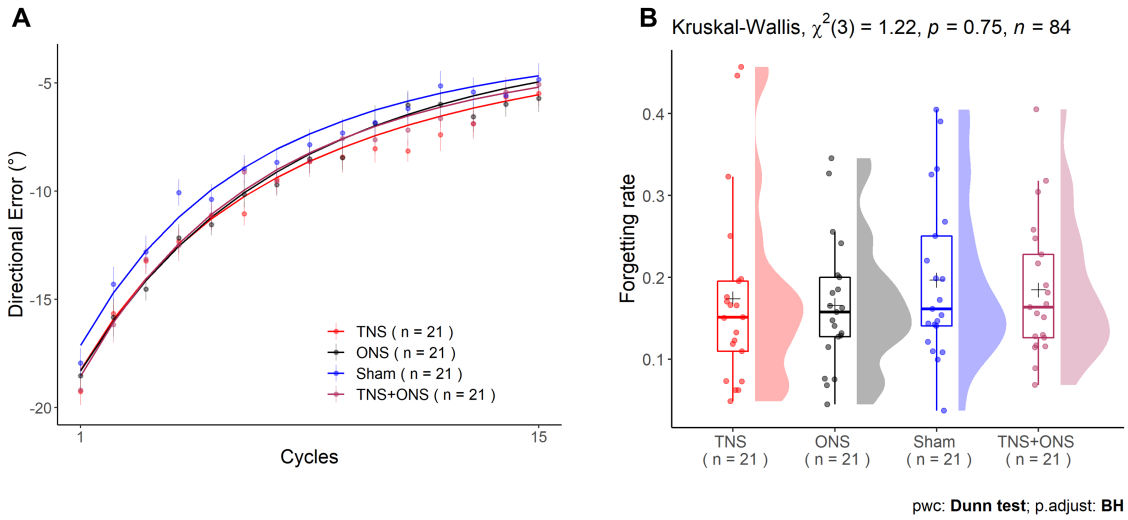

Figure 6.8: A) Forgetting curve. B) Forgetting rates comparison.

experienced discomfort in the forehead belonged to ONS+TNS (2) and ONS (1). The ONS+TNS participants received 9.1 mA and 9.8 mA, and gave a discomfort level of 1 and 5, respectively. On the other hand, the ONS participant complained during the sham-TNS period. She immediately reported discomfort when the stimulation was on. Thus, the fixed threshold of 6 mA was reduced to 5.1 mA for the remainder of the sham-TNS (60 seconds), which was well-tolerated.

Additionally, subjects who belonged to the active groups reported tingling and pressure in the forehead and back of the head during the stimulation. Also, a small percentage of participants reported to have experienced a headache (TNS: n=1, rate=4/10; ONS: n=4, rate=5/10 ±2.4; TNS+ONS: n=3, rate=3.7/10 ±2.5; Sham: n=2, rate=3.5/10 ±0.7), dizziness (TNS: n=3, rate=4.7/10 ±0.6, ONS: n=1, rate=2/10; TNS+ONS: n=3, 4.3/10 ±3.2; Sham: n=1, rate=3/10), blurry vision (TNS: n=2, rate=3.5/10 ±0.7; ONS: n=4, rate=5.3/10 ±2.1; TNS+ONS: n=1, rate=1/10; Sham: n=1, rate=2/10) and skin irritation at the electrode site (TNS: n=1, rate=3/10; ONS: n=2, 4.5/10 ±0.7; TNS+ONS: n=2, rate=2/10 ±0; Sham:

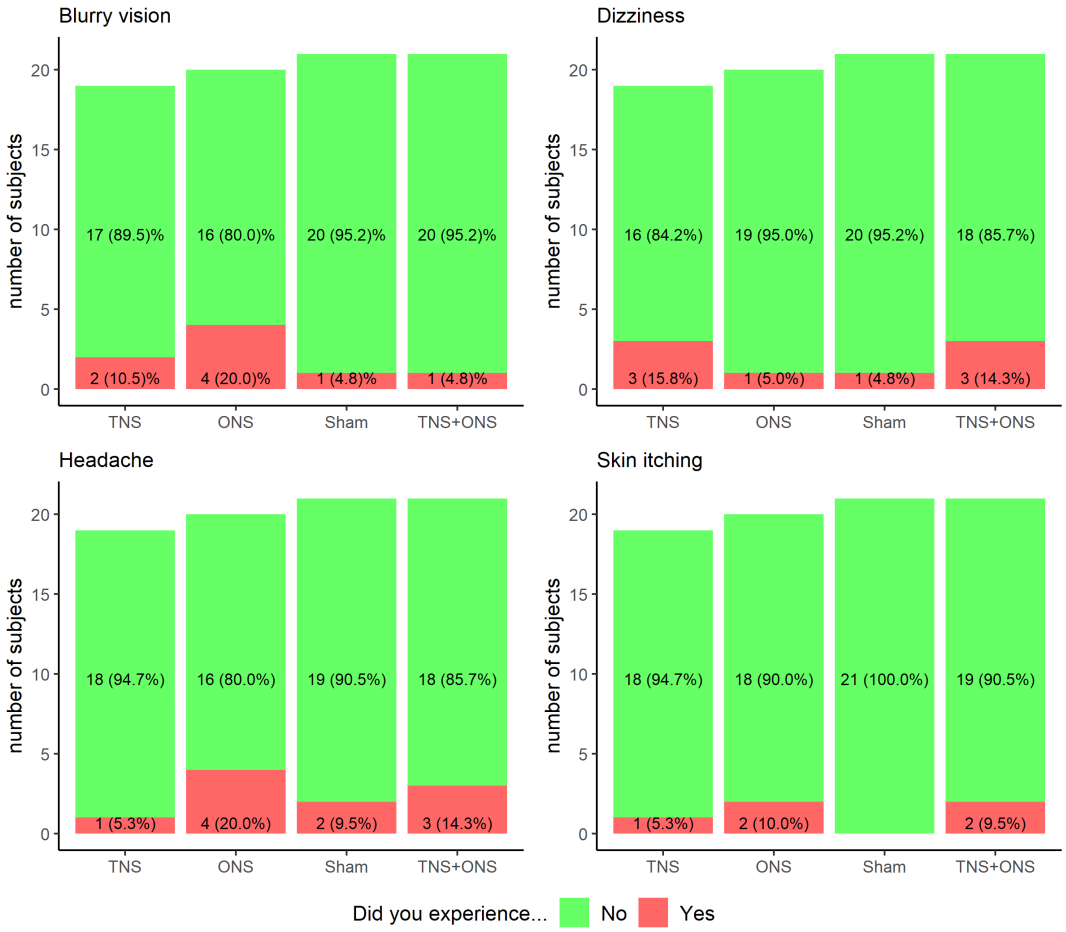

Figure 6.9: Subjects were consulted for blurry vision, dizziness, headache and skin itching at the electrodes position during or after the experiments.

n=0) during or after the experiment. Fig. 6.9 summarize resume these results.

Sham Analysis

Within the sham group, 47.6% (10 out of 21) of the participants believed they received stimulation. The sham approach involved 60 seconds fixed current, which was different from experiments 1A, 1B, and 2, where no current was delivered. From this subgroup, 50.0% (5 out of 10) believed their motor performance improved after the stimulation. We examined the learning curves for these subgroups of the sham group, to assess some potential placebo effects. In this case, we split the sham into three

groups: (1) participants who felt the stimulation and perceived motor improvement (***Sh-felt-imprv***, n=5) , (2) participants who did not feel improvement despite feeling the simulation (***Sh-felt-no-imprv***, n=5), and (3) participants who did not feel the stimulation at all (***Sh-no***, n=11). Fig. 6.10.A illustrates the learning curves for the sham and its subgroups, as well as the ONS group, which had the fastest improvement. It is possible to observe that individuals who experienced the stimulation and perceived improvement exhibited rapid learning at a similar rate to those in the ONS group. On the other hand, participants who did not perceive any improvement showed the slowest learning. Those who did not feel the stimulation showed a similar rate of learning than the overall sham group.

Considering that ***Sh-felt-imprv*** group's improvement can be attributed to a placebo effect, we excluded this subgroup from the sham group. Consequently, recalculating the learning curves (Fig. 6.10.B) resulted in all the active groups outperforming the sham group.

Furthermore, the active sham learning curve was compared with the passive sham from previous experiments. Fig. 6.11 shows that the learning curves for sham group in experiments 1A, 1B, and 2 (blue lines) overlap, suggesting a similar learning rate for all of them. On the other hand, the active sham group from experiment 3 adapted to the rotation faster than the passive sham during the early part of the adaptation, but then converged with the passive sham in the late part of the adaptation. This comparison suggests a potentially stronger placebo effect in the active sham group than the passive sham group.

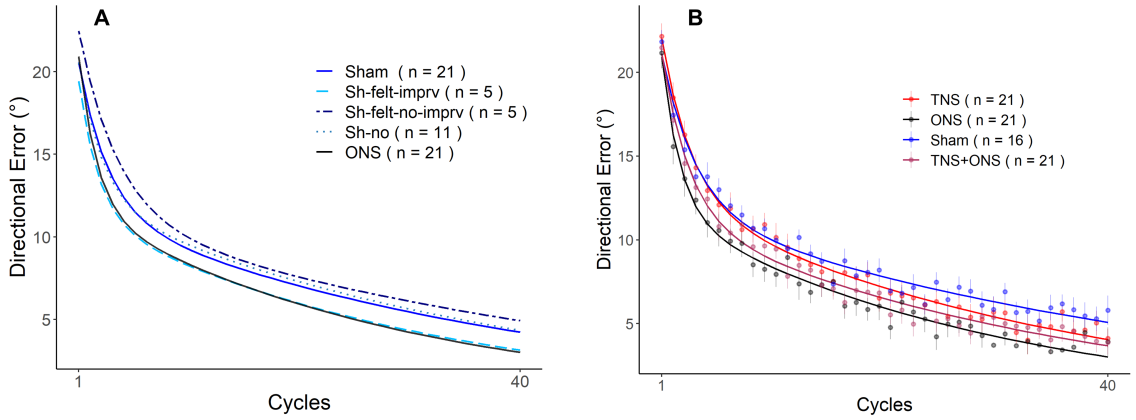

Figure 6.10: A) Sham group split into participants who perceived the stimulation and improvement in motor performance (Sh-felt-imprv), who perceived the stimulation but no motor improvements (Sh-felt-no-imprv), and who did not perceive the stimulation at all (Sh-no) B) Sham learning curve after the removal of the subgroup Sh-felt-imprv compared to active groups.

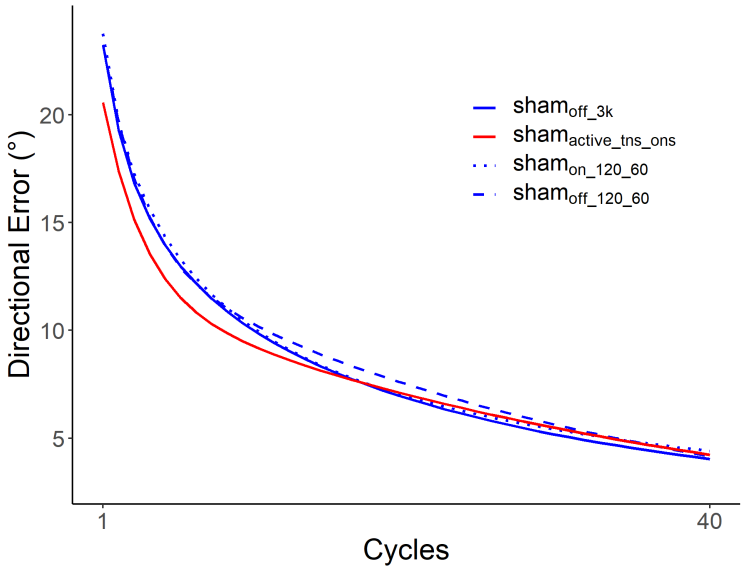

Figure 6.11: Learning curves for all the passive sham interventions in experiments 1A, 1B, and 2 (blue line) and the active sham approach (red line).

6.8 Discussion

This study provided preliminary information regarding the effects of TNS and ONS on visuomotor learning using kilohertz range frequencies. We aimed to compare both separately, but also in combination to explore a potential synergistic effect due to the convergence of the trigeminal and occipital nerves toward brain stem nuclei.

Effects on Learning Rates

Analyses of learning curves revealed that the groups exposed to ONS demonstrated the fastest learning rates among groups. However, learning coefficients comparison did not confirm the effect. Surprisingly, the learning rates for TNS did not appear to be substantially faster than those of the sham group, in contrast to the results presented in section 4.7.2, where it showed rapid learning rates compared to a passive sham (no current delivered). Moreover, subjects who received concurrent stimulation learned faster than the sham and TNS groups but slower than the ONS alone, suggesting that there are no synergistic effects using this protocol as initially hypothesized. These results suggest that ONS may have more beneficial effects on motor learning than TNS.

There are some factors that might explain the differences between modalities. For instance, the electrode size. ONS was delivered using soaked-saline sponges that allows stimulation through the hair as usually done in tDCs studies. These sponges had a larger round shape with a diameter of 3.81 cm, whereas the TNS electrodes had a diameter of 3.2 cm. This difference in size might have influenced the participant's ability to tolerate current. On average, the ONS group tolerated higher intensities (14.0±2.8 mA) than TNS (7.2±2.6 mA), resulting in higher current densities for ONS (1.228 mA/cm_2) than TNS (0.895 mA/cm_2). The higher amount of current and

density delivered for ONS might have contributed to the observed effects.

Furthermore, larger electrodes can result in less focalized effects, potentially leading to the spread of current to other areas such as the cerebellum. It is well known the importance of the cerebellum in learning, and studies using cerebellar tDCs have shown affecting learning rates in a visuomotor learning task [67]. While our study has focused on the ability of ONS to activate bottom-up mechanisms, we cannot dismiss the possibility we might have indirectly stimulated the cerebellum through a transcranial mechanism. In [46], they investigate the effects of tDCs over the occipital nerve on memory improvement. To determine whether the observed effects were mediated by transcranial or transcutaneous mechanisms, they blocked the nerve using a skin anesthetic. They observed that the anesthetized group still show improvements compared to sham, although not to the same extent as without anesthesia. This suggests that some current might reach the brain and produce a cortical effect. In tDCs studies, the current is approximately 2 mA, significantly less than the average used in our study. Although, we used biphasic pulses with a 50 μs pulse width, instead of direct current, the use of higher intensities might still contribute to transcranial effects.

Sham Groups Might be Affected by Placebo Effects

Based on the findings from experiments 1A, 1B, and 2, it was consistently observed that subjects from the sham group who perceived the stimulation performed at a rate similar to the faster performers. This experiment was not the exception. Participants who reported feeling and perceiving motor improvement due to the stimulation learned the rotation at a similar rate compared to ONS. However, individuals who felt the stimulation but did not perceive any improvement presented impaired learning.

Thus, assuming the overall performance of the sham group was affected by a

placebo effect, its learning curve was recalculated by excluding subjects who felt the stimulation and perceived motor improvement. When these participants were removed, all active groups, including TNS, performed faster than the sham. This approach allows controlling factors such as perception of receiving the stimulation and improvement of performance which might influence learning behavior.

In this experiment, an active approach was used, where participants received 60 seconds of stimulation to reinforce blinding which has been used in tDCs studies [94]. Interestingly, when comparing the learning profile of the active sham group with the passive sham approach used in experiments 1A, 1B, and 2, it was observed that the active sham exhibited faster learning in the initial part of the adaptation compared to the passive sham. One possible explanation for this improvement in the active sham group could be attributed to a stronger placebo effect resulting from the combination of sensations produced by the brief stimulation and the more extensive setup used for this study. The experimental setup included the placement of electrodes on the forehead and at the back of the head, along with a rubber band to hold the electrodes in the back. Subjects described sensations of pulsation, wetness (due to the saline), and tightness due to the presence of the rubber band and electrodes in the back even before the stimulation began. The combination of these sensations, coupled with the 60-second stimulation may have intensified the overall placebo effect observed in the previous studies.

Alternatively, although it is unlikely, another possible explanation for the improvement observed is that the brief stimulation might have triggered some neurobiological changes that modulate learning. This is something that has been discussed in the tDCs field, where placebo effects have been reported. For instance, in [95], both sham and actual tDCs were able to modulate EEG parameters such as P3 associated with working memory, suggesting that sham-tDCs might also alter neural processes.

Therefore, despite the small duration of 60 seconds compared to the active group, the potential effects of sham stimulation cannot be dismissed, and further research is necessary to assess their effects.

This series of observations suggest that perception of stimulation and improvement of performance might play a role in learning. Consequently, unmasking the true effects of stimulation interventions becomes a challenge. For this reason, it is crucial to control these intrinsic factors. This way, researchers can have more tools to identify the effects of stimulation.

Stimulation Tolerability

Inquiring the subjects about the location of the discomfort allowed us to determine whether the discomfort was produced by the stimulation or fatigue in the arm due to the repetitive task. In this experiment, 17.3% of all participants (14 out of 81) reported discomfort. From this subgroup, 78.6% of the cases were due to pain in the arm or shoulder. As suspected, the cause of discomfort during the experiment was associated with fatigue in the arm and shoulder due to the multiple reaching movements performed for almost two hours. However, three participants (21.4%) experienced discomfort in the forehead associated with TNS, which was resolved by reducing the stimulation intensity. It is important to note that participants selected a threshold that they considered comfortable and could reduce the current at any time. Thus, extrapolating these findings to experiments 1A, 1B, and 2, it was likely the reported discomfort was associated with arm fatigue.

In general, participants tolerated well the stimulation. There was only one case of lightheadedness after two minutes of TNS, similar to the case described in 1B, where 3 kHz TNS was also used. Unfortunately, this subject also did not disclose their history of fainting episodes. Additionally, participants were consulted specifically for

side effects such as blurry vision, dizziness, headaches, and skin itching. For ONS, the most commonly reported was headache (4 out of 20, 20%) and blurry vision (4 out of 20, 20%). TNS as well as TNS+ONS groups reported dizziness in three cases, equivalent to approximately 15% of participants of each group. The occurrence of these adverse events was similar to experiment 1B and in line with what has been reported in the VNS and TNS studies [13, 96].

6.9 Conclusion

In this study, our goal was to compare the effects of TNS with ONS. We observed pronounced learning with ONS during the rotation block of a visuomotor learning task; however, no statistically significant differences were found. Furthermore, we explored ONS and TNS applied concurrently, aiming to investigate potential synergistic effects on learning, but our data did not provide support for this hypothesis. Despite this, we observed some interesting trends. For instance, the ONS group had rapid learning among groups. Factors that might have contributed to these effects were the higher intensities tolerated compared to TNS and potential transcranial effects that might have activated cerebellar regions, which is well known for their influence on learning mechanisms.

We also observed a potential placebo effect in our sham group when we analyzed a subgroup of subjects who reported having experienced the stimulation and perceived motor improvement. This subgroup presented a learning rate similar to the ONS, which was the fastest curve. Recalculating the learning curves without these participants it was shown that all the active groups were faster than the sham. Interestingly, when TNS was compared with the overall sham, it did not replicate the results shown in experiment 1B, but when compared with the recalculated sham, shows similar results as our previous experiment. Thus, finding better ways to control placebo effects

which have been described as strong in studies that consider devices like in the brain stimulation field will help to clear the true effect of these stimulation protocols.

Chapter 7

DISCUSSION

7.1 Summary and Key Takeaways

These series of studies provide preliminary data regarding the effectiveness of single-session transcutaneous electrical stimulation of the trigeminal and occipital nerves on visuomotor adaptation in healthy adults. These results will serve as a foundation for future studies designed to explore the feasibility of using these neuromodulatory techniques as an adjuvant to conventional neurorehabilitation of sensorimotor dysfunction. More generally, the information obtained from this study serves as a starting point for efforts aimed at developing optimal Trigeminal Nerve Stimulation (TNS) and Occipital Nerve Stimulation (ONS) parameters and protocols for a) improving sensorimotor performance in older adults, b) augmenting conventional neurorehabilitation, and c) enhancing human sensorimotor performance in the industrial, athletic, military, and performing arts settings.

7.1.1 TNS and ONS Effects on Learning

We provided preliminary evidence that TNS can modulate the rate at which a motor task can be learned. Offline TNS using a 60 Hz frequency slowed down the learning of a visuomotor rotation task, compared to sham TNS. This behavioral observation might be explained by the ability of TNS at 60 Hz to alter high or low levels of tonic firing of neurons in the Locus Coeruleus (LC), which has been shown to be associated with poor performance [84]. On the other hand, when TNS at 60 Hz was delivered online, we observed much faster learning compared to offline TNS using the

same frequency. This timing-dependent effect suggests different neural mechanisms may be at play during offline vs online TNS. Although Vagus Nerve Stimulation (VNS) has been shown to alter LC activity within milliseconds in animal models [16], fMRI studies in humans designed to assess brain areas activated by Transcutaneous Auricular Vagus Nerve Stimulation (taVNS) have shown that maximum activation of the Nucleus Tractus Solitarius (NTS) and other brain regions happened several minutes post-stimulation [97]. This suggests that the activation of brain stem nuclei and subsequent neurotransmitter release is a more suitable mechanism for offline stimulation. On the other hand, the effects produced by online stimulation might then be explained by a different mechanism, i.e., increased cortical excitability, which has been postulated to underlie transcranial effects. In support of this idea, simulation studies have suggested that part of the current applied by supraorbital TNS can pass to frontal areas and affect cortical excitability driving behavioral changes [22].

On the other hand, participants that received offline 3 kHz TNS showed more rapid learning compared to those receiving sham TNS. Similar to the rationale for offline 60 Hz TNS, it is possible that 3 kHz TNS might have optimized LC activity by facilitating phasic LC firing, which has been linked to optimal performance. It is important to note that while the results of our studies employing 60 Hz TNS supports stimulation and timing-dependent effects, neither online nor offline stimulation at 60 Hz resulted in significantly faster learning than sham stimulation. In contrast, participants receiving the offline 3 kHz protocol demonstrated significantly faster learning than those receiving the sham protocol, suggesting it is a better candidate for TNS modulation. However, effects on learning were still relatively modest. Future research should therefore focus on parameter optimization, with the goal of facilitating stronger effects.

We also compared 3 kHz TNS with 3 kHz ONS, given the convergence between

these two nerves, and potential sharing of mechanisms of action. Although we did not find differences between groups, we observed some interesting trends that suggest that ONS might be more suitable for enhancing learning. ONS showed the fastest adaptation rate among groups. Additionally, we explored TNS and ONS combined, expecting to see more pronounced effects than what was observed with the individual modalities. However, TNS+ONS resulted in more rapid learning than TNS and sham but showed slower learning rates than ONS, suggesting no synergistic effects using this protocol. Surprisingly, TNS was associated with the slowest learning rates among active groups, which was unexpected due to the encouraging results observed in experiment 1B, and was even associated with slightly lower learning rates than sham. To explore this surprising result, we asked subjects in the sham group whether they believe they received stimulation and, if so, whether they experienced performance improvement. Interestingly, a subgroup of participants that perceived and experienced performance improvement due to the stimulation showed similar learning rates compared to the fastest group, suggesting a placebo effect. Removing this subgroup from the overall sham resulted in faster learning for TNS, similar to the results shown in experiment 1B.

Despite the evidence provided that TNS and ONS affects motor learning behavior, the magnitude of the observed effects were modest. These modest effects may represent a floor effect on visuomotor learning in healthy young adults. However, recent work suggests that the degree of visuomotor adaptation is reduced and more variable in older adults. Thus, a potentially fruitful avenue for future investigations could involve examining an older population with the same paradigm. This way, a better understanding of TNS and ONS's potential to ultimately enhance neuroplasticity and visuomotor learning in 'age-matched' adult stroke subjects could be obtained [70].

7.1.2 TNS and ONS Safety and Tolerability

Additionally, these experiments provide valuable information regarding TNS and ONS safety and tolerability for a single session of approximately 20 minutes duration (10 min effective). We showed that by using Kilohertz Electrical Stimulation (KES), the submaximal tolerable threshold reaches higher intensities and can be delivered safely. Adverse events were in line with what has been reported in the literature where headaches and dizziness were the most common complaints but were still observed in only a relatively small percentage of participants. In only 2 cases, subjects felt lightheaded, and the stimulation was stopped. However, in both cases, participants did not disclose previous fainting episodes which were part of the exclusion criteria. Thus, fainting and vaso-vagal syncope episode conditions should be continue to be excluded to avoid these episodes.

7.2 Remaining Questions, Limitations, and Future Work.

In these experiments, several stimulation parameters were tested, which to our judgment, provided good starting points to assess the feasibility of the effects of TNS on motor learning. However, these parameters are a minimal sample of the overall parameters space. For example, we addressed the effects of a single session of approximately 10 minutes of effective stimulation, which, as described above, produced modest effects. Therapeutics effects observed in TNS have been shown to require weeks or months of treatment [23]. Therefore, increasing the session length or applying multiple sessions across several days might enhance the efficacy of TNS. Moreover, we utilized cycled stimulation (30 seconds ON/OFF) because it was commonly used in the TNS literature for Attention-Deficit/Hyperactivity Disorder (ADHD) and Drug-Resistant Epilepsy (DRE). However, TNS studies for migraine have used continuous

stimulation with equally beneficial effects. For this reason, further investigation on continuous stimulation could provide additional valuable information on TNS efficacy. Finally, we also compared offline vs online stimulation for the 120 and 60 Hz groups. However, we did not address 3 kHz delivered online, which may provide further insights into TNS efficacy. Furthermore, future work should consider pairing the stimulation with movements, which have shown to be effective for rehabilitation using VNS [2].

In all experiments, the stimulation intensity was tuned to a self-selected submaximal tolerable threshold. We adopted this approach because in the majority of TNS studies, the current have been determined by the study participants themselves. Also, TNS have been prescribed as an at home-therapy for migraine and ADHD, offering participants more control to the dose they received. Although the mean current delivered between groups with similar frequencies were not statistically significant different from each other, this approach might have introduced variability to the results that led to modest effects. In addition, we explored potential correlations between intensities and the learning rates but no clear trends were found. An alternative approach could have been used a fixed current for all subjects, similar to the approach used in the Transcranial Direct Current Stimulation (tDCS) literature where a fixed intensity of 2 mA is usually applied . Exploring whether fixed vs self-selected intensities yield more favorable results will require further investigation.

All conducted experiments were sham-controlled. In experiments 1A, 1B and 2, a passive sham approach was used, where electrodes were placed on the same placed as the active groups but no current was delivered. However, in experiment 3, we applied a fixed current during 60 seconds similar to others studies. We observed that perception of receiving the stimulation affected learning in a positive way, suggesting a placebo effect. Having an additional control group where no electrodes are involved

and participants only performed the task, would help to quantify the magnitude of these placebo effects and provide a better assessment of the stimulation groups.

A visuomotor rotation task was used to study adaptation, a form of motor learning where known movements need to be adapted due to a perturbation in the environment. This task have been extensively studied, and it has been shown that some participants with a characteristic skill set, such as athletes [98] and minimally invasive surgeons [99], might learn the perturbation at a faster pace than a non-expert control. On the other hand, people with specific genetic profiles, such as a polymorphism of the Brain-derived neurotrophic factor gene, might affect the rate of learning compared to a control group [100]. Futures studies using this task should take these factors into consideration. Additionally, many other tasks fall under the motor learning category that could be assessed such as the serial reaction time task, among others. Moreover, other clinically relevant tasks might need to be explored in populations with motor learning deficits such as older adults and, neurologically-impaired individuals, which could reveal bigger larger effects of stimulation.

In this work, we did not focus our attention on the neural mechanisms involved in these techniques. One of the main questions pending is whether these effects are mediated purely via transcutaneous, transcranial, or both mechanisms. That is, although we focused on these techniques because of their novel approach to stimulating bottom-up pathways, we cannot discard the possibility that protocols utilized in these experiments also affect the cortex via transcranial mechanisms. There is some evidence based on simulation studies suggesting that stimulation of the supraorbital branches of the trigeminal nerve might also activate frontal areas. Additionally, studies using tDCS over the occipital nerve have shown residual behavioral effects even after blocking the nerve. This suggest that some of the behavioral effects of ONS result via transcranial mechanisms. To clear up whether transcranial, transcutaneous

or a combination of both mediate the observed effects, further studies might consider blocking either the trigeminal or the occipital using skin aesthetic as done in [5] to control for peripheral contributions, and this way, track the source of the effects.

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APPENDIX A

EXCLUSION CRITERIA

Exclusion Criteria
• Left-handed. • Age younger than 18 years old. • Treatment or medication for neurological or psychological disorder, including addiction. • Medical Implants. • Panick attack or acute anxiety disorder. • Frequent fainting, vaso-vagal syncope, or neurocardiogenic syncope (even once). • Raynaud's disease. • Temporomandibular joint disorder or facial neuropathy. • Significant face/head injury or having any cranial or facial metal plate or screw implants. • Concussions or brain injury. • Vision or hearing impairment that is uncorrectable. • Current pregnancy. • Recent drug or alcohol treatment (within past 3 months). • High Blood pressure and heart disease (including rhythm disturbances such as palpitations, Wolf-Parkinson-White syndrome etc.) • Diabetes

Table 1: Exclusion Criteria.

APPENDIX B

EDINBURGH HANDEDNESS INVENTORY - SHORT FORM

Edinburgh Handedness Inventory - Short Form

Please indicate your preferences in the use of hands in the following activities or objects:

	Always right	Usually right	Both equally	Usually left	Always left
Writing	☐	☐	☐	☐	☐
Throwing	☐	☐	☐	☐	☐
Toothbrush	☐	☐	☐	☐	☐
Spoon	☐	☐	☐	☐	☐

Scoring:

For each item: Always right = 100; Usually right = 50; Both equally = 0; Usually left = -50; Always left = -100

To calculate the Laterality Quotient add the scores for the four items in the scale and divide this by four:

Writing score	
Throwing score	
Toothbrush score	
Spoon score	
Total	
Total ÷ 4 (Laterality Quotient)	

Classification:	Laterality Quotient score:
Left handers	-100 to -61
Mixed handers	-60 to 60
Right handers	61 to 100

APPENDIX C

IRB APPROVAL

ASU Knowledge Enterprise Development

APPROVAL:CONTINUATION

Christopher Buneo
BHSE: Biological and Health Systems Engineering, School of
480/727-0841
Christopher.Buneo@asu.edu

Dear Christopher Buneo:

On 8/26/2020 the ASU IRB reviewed the following protocol:

Type of Review:	Modification and Continuing Review
Title:	Transdermal electric nerve stimulation (TENS) for enhancement of upper extremity visuomotor function
Investigator:	Christopher Buneo
IRB ID:	STUDY00006393
Category of review:	
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• Post_study_questionnaire.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Arias Certification Training.pdf, Category: Other; • Buneo Certification Training.pdf, Category: Other; • Consent.pdf, Category: Consent Form; • HRP-503b-TEMPLATE_PROTOCOLBioscience.docx, Category: IRB Protocol; • Lab safety plan approval - Research Intensification Plan_ Summer 2020.pdf, Category: Other; • recruitment_email.pdf, Category: Recruitment Materials; • Script.pdf, Category: Recruitment Materials;

The IRB approved the protocol from 8/26/2020 to 8/25/2021 inclusive. Three weeks before 8/25/2021 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 8/25/2021 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
Justin Pettit
William Tyler
Christopher Buneo
Diego Arias Velasquez

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Christopher Buneo
IAFSE-BHSE: Bioengineering, Harrington Department of
480/727-0841
Christopher.Buneo@asu.edu

Dear Christopher Buneo:

On 8/31/2022 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Effects of transdermal electric nerve stimulation (TENS) on visuomotor function
Investigator:	Christopher Buneo
IRB ID:	STUDY00016419
Category of review:	
Funding:	None
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• ASU Student Recruitment_Data Collection (Buneo, STUDY00016419, TENS enhanced visuomotor function) -- Approved.pdf, Category: Other; • Consent_aiming.pdf, Category: Consent Form; • Consent_reaction_time.pdf, Category: Consent Form; • Eligibility_and_Health_Survey.pdf, Category: Screening forms; • Post_study_questionnaire_aiming.pdf, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Recruitment_email_aiming.pdf, Category: Recruitment Materials; • Recruitment_email_reaction_time.pdf, Category: Recruitment Materials; • Recruitment_flyer_aiming.pdf, Category: Recruitment Materials; • Script_and_Instructions_aiming.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • Script_reaction_time.pdf, Category: Recruitment materials/advertisements /verbal scripts/phone scripts; • TENS Enhanced Visuomotor Function.docx, Category: IRB Protocol;

The IRB approved the protocol from 8/31/2022 to 8/30/2023 inclusive. Three weeks before 8/30/2023 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 8/30/2023 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

REMINDER - - Effective January 12, 2022, in-person interactions with human subjects require adherence to all current policies for ASU faculty, staff, students and visitors. Up-to-date information regarding ASU's COVID-19 Management Strategy can be found here. IRB approval is related to the research activity involving human subjects, all other protocols related to COVID-19 management including face coverings, health checks, facility access, etc. are governed by current ASU policy.

Sincerely,

IRB Administrator

APPENDIX D

PERMISSION FROM SCIENTIFIC JOURNAL

Some of the procedures and results reported in Experiment 1B were published in the Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society under the following citation:

- D. E. Arias and C. A. Buneo, Effects of Kilohertz Electrical Stimulation of the Trigeminal Nerve on Motor Learning, Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. EMBS, vol. 2022-July, pp. 5103–5106, 2022.

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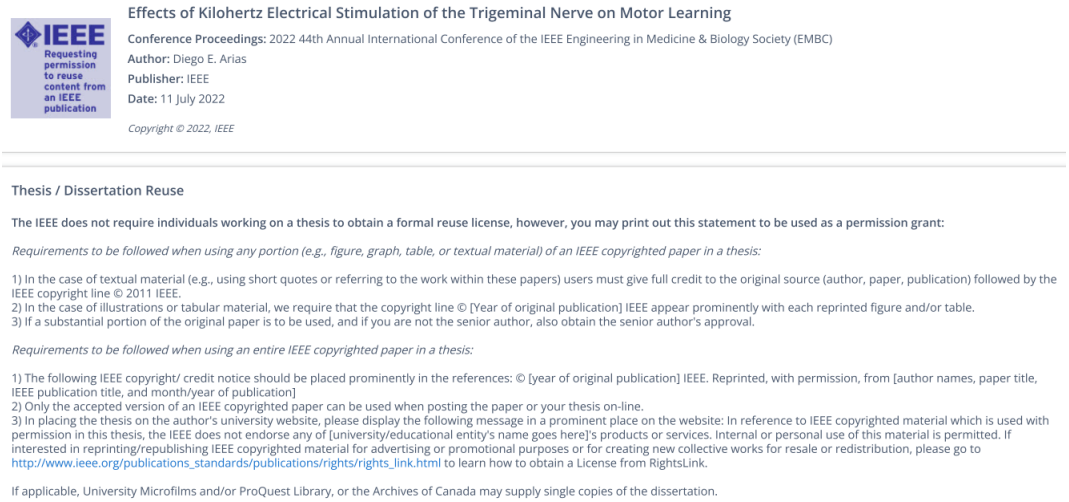

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