

**IDENTIFYING THE NEURAL CORRELATES OF MOTOR SEQUENCE LEARNING
AND MOVEMENT AUTOMATICITY**

By

Nadia Polskaia

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School of Human Kinetics

Faculty of Health Sciences

University of Ottawa

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Abstract

Sequential movements have become a common experimental paradigm for evaluating the neural correlates of motor learning. Currently, the understanding is that motor sequence learning engages the cortico-cerebellar and cortico-striatal networks and that their contributions differ depending on the stage of learning. The prefrontal cortex (PFC), in particular, has been observed at the early/fast phase and late/slow phase of motor sequence learning, suggesting involvement in processes such as movement automaticity, stimulus-response conflicts, explicit learning, and retrieval, to name a few. However, it is difficult for neuroimaging studies to evaluate the relative permanence of motor sequence learning due to the financial burden associated with functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). In this dissertation, four experiments were performed to examine the functionality of functional near-infrared spectroscopy (fNIRS) in elucidating the role of the PFC in motor sequence learning and movement automaticity.

The first experiment (Chapter 2) focused on validating fNIRS as a comparable technique to fMRI by replicating a previous motor sequence learning study (Wu et al., 2004) that reported decreased activity in the left dorsolateral prefrontal cortex (DLPFC) following achievement of movement automaticity. The fNIRS findings were unable to detect a similar decrease in the left DLPFC.

The second experiment (Chapter 3) further investigated the cerebral oxygenation changes in the PFC following motor sequence learning. To enable better distinction between learning and performance, this experiment including multiple motor sequence tasks, a control group, four practice sessions and a retention phase. The findings revealed increased contributions from the right hemisphere (e.g., right ventrolateral PFC (VLPFC)) and the suggestion that the left DLPFC

may not reflect movement automaticity but rather attentional investment in movement preparation.

To address the signal processing concerns observed in experiment one and two, the third experiment (Chapter 4) investigated the effect of five motion correction techniques on the statistical outcomes of a motor sequence learning experiment. Additionally, the corrections were evaluated to determine which would yield the greatest improvement in hemodynamic response function (HRF) recovery and within-subject standard deviation. The findings revealed the location of significance to vary depending on the motion correction applied. Also, wavelet and spline + wavelet demonstrated limited improvement in reducing within-subject standard deviation.

Lastly, the fourth experiment (Chapter 5) examined changes in the PFC associated with dual-task processing before and after motor sequence learning. Findings revealed decreased activity in the right DLPFC, medial PFC (mPFC), and orbitofrontal cortex following practice for dual-task sequence-4. A similar but marginal trend was observed in the right VLPFC. Minimal significance was observed during the dual-task sequence-12 task.

Collectively, the findings of this dissertation suggest that 1) motor sequence learning when acquired with explicit knowledge requires contribution from predominately the right hemisphere, 2) the left DLPFC may represent attentional investment in movement preparation rather than movement automaticity, 3) the neural representations of dual-task processing are associated with the complexity of the motor sequence task, and 4) low-frequency motion artifacts may be difficult to remove using certain signal processing methods.

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Glossary of Terms

PFC: Prefrontal cortex

DLPFC: Dorsolateral prefrontal cortex

VLPFC: Ventrolateral prefrontal cortex

mPFC: Medial prefrontal cortex

fNIRS: Functional near-infrared spectroscopy

HbO: Oxyhemoglobin

HbR: Deoxyhemoglobin

tHb: Total hemoglobin

HRF: Hemodynamic response function

S4: Sequence-4

S12: Sequence-12

S1: Sequence-1

S2: Sequence-2

S3: Sequence-3

S4: Sequence-4

ST: Single-task

DT: Dual-task

SRTT: Serial reaction time task

PCA: Principal component analysis

CBSI: Correlation-based signal improvement

CNR: Contrast-to-noise ratio

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I, Nadia Polskaia, was responsible for the concept and experimental design of all experiments, with input and guidance from Dr. Yves Lajoie (Supervisor) and my thesis committee comprised of Dr. Nicole Paquet and Dr. Sarah Fraser. Gaby St-Amant assisted in participant recruitment and data collection (Experiments 2, 3, and 5). Dr. Yves Lajoie assisted in the design and set-up of the experimental protocols (Experiments 2, 3, and 5). Dr. Sarah Fraser provided the functional near-infrared spectroscopy (fNIRS) device for all experiments. Data and statistical analysis was performed primarily by me, with assistance from Dr. Yves Lajoie. Preparation of all manuscripts was completed by me, with contributions from Dr. Yves Lajoie, Dr. Sarah Fraser, and Gaby St-Amant.

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1. General Introduction

The ability to acquire new knowledge and motor skills is an essential aspect of human life. The process of motor learning has incited considerable interest among researchers over the years (see Kantak & Winstein, 2012 for a review). It is often described as a relatively permanent change in the capability to perform a skilled movement as a result of practice or experience (Schmidt & Lee, 2011). It involves changes in neural, sensory, and cognitive processes that cannot be directly observed and, therefore, must be inferred from changes in motor behaviour (Kantak & Winstein, 2012; Schmidt & Lee, 2011). Sequential movements have become an important experimental paradigm for understanding the neural correlates of motor learning (see Lohse, Wadden, Boyd, & Hodges, 2014 for a review) and will be the focus of this dissertation.

Motor sequence learning is defined as the acquisition of a series of pre-determined motor actions through practice (Dahmns, Brodoehl, Witte, & Klinger, 2019; Doyon, 2008; Penhune & Steele, 2012). It is generally agreed that it engages the cortico-cerebellar and the cortico-striatal networks, and that their contributions tend to differ depending on the stage of learning (Lohse et al., 2014). For the purpose of this dissertation, the prefrontal cortex (PFC) will be the only region of interest. Neuroimaging studies have observed increased PFC activation in the early/fast phase and late/slow phase (consolidation and automatization) of motor sequence learning (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Doyon et al., 2002; Eliassen, Souza, & Sanes, 2001; Grafton, Hazeltine, & Ivry, 1995; Hazeltine, Grafton, & Ivry, 1997; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Jueptner et al., 1997b; Penhune & Doyon, 2002; Poldrack et al., 2005; Toni, Krams, Turner, & Passingham, 1998; Wu, Kansaku, & Hallett, 2004). As a result, the PFC has been implicated in movement-related processes (e.g., automaticity, stimulus-

response conflicts, etc.) and working memory functions (e.g., explicit learning, memory retrieval, etc.), respectively.

However, it is difficult for neuroimaging studies to evaluate the relative permanence of motor sequence learning due to the high cost associated with neuroimaging (e.g., fMRI and PET). Functional near-infrared spectroscopy (fNIRS) is a neuroimaging technique that can enable long-term evaluation due to its low cost and portability ((Bunce, Izzetoglu, Izzetoglu, Onaral, & Pourrezaei, 2006; Quaresima & Ferrari, 2019). fNIRS provides an indirect and non-invasive method for monitoring cerebral oxygenation changes associated with the neurovascular coupling response (Perrey, 2008). It can quantify the relative concentration of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) by capturing changes in light intensity in the near-infrared range (700 – 900 nanometers (nm)) (Bunce et al., 2016; Perrey, 2008; Scholkmann et al., 2014). Over the last 25 years, the rapid growth of fNIRS research (Boas, Elwell, Ferrari, & Taga, 2014) has produced a number of pre-processing methods aimed at improving signal quality and hemodynamic response recovery, however, there is still no consensus or standardized processing method (Hocke et al., 2018; Pinti et al., 2018). This can lead to suboptimal studies, misinterpretations, and irreproducibility (Hocke et al., 2018; Pinti, Scholkmann, Hamilton, Burgess, & Tachtsidis, 2019). Therefore, the overall goal of this dissertation was to examine the capability and limitations of fNIRS in elucidating the role of the PFC in motor sequence learning and movement automaticity.

1.1. Motor sequence learning

Motor sequence learning refers to the process by which a series of pre-determined motor actions are performed through practice (Dahmns et al., 2019; Doyon, 2008). This enables motor

parameters to be optimized, resulting in accurate and efficient performance (Penhune & Steele, 2012). The process of motor sequence learning is thought to follow three distinct phases: an early learning phase, a consolidation phase, and a slow learning or retention phase (Dahms et al., 2019; Doyon & Benali, 2005; Penhune & Steele, 2012). In the early phase, rapid improvement to performance is observed, typically within the first practice session. These changes are usually caused by improved encoding of the spatial coordinates associated with the movements (Dahms et al., 2019; Hikosaka, Nakamura, Sakai, & Nakahara, 2002). In the consolidation phase, performance stabilizes and becomes increasingly resistant to interference (Dahms et al., 2019; Doyon & Benali, 2005; Penhune & Steele, 2012). This stage is thought to be heavily reliant on sleep (Fischer, Hallschmid, Elsner, & Born, 2002; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). In the slow stage, also known as the automatization phase, performance is optimized, and the sequential movements can be performed with little to no attentional demand (Penhune & Steele, 2012).

Motor sequence learning can occur with or without conscious awareness (explicit vs. implicit learning) (Dahms et al., 2019). Explicit learning involves the conscious recollection of previous events (Honda et al., 1998). Implicit learning refers to behavioural improvements that occur without awareness, as a result of practice (Honda et al., 1998; Rose & Christina, 2006). It has been suggested that explicit and implicit learning are independent of one another during motor sequence learning (Curran & Keele, 1993; Nissen & Bullemer, 1978; Nissen, Knopman, & Schacter, 1987; Willingham, Nissen, & Bullemer, 1989). In the classic example by Nissen and Bullemer (1987), participants with memory impairment were able to successfully learn a serial reaction time task (SRTT), despite having a compromised declarative memory. Similar findings were observed in healthy subjects (Willingham et al., 1989), under dual-task conditions (Curran

& Keele, 1993), and with the administration of scopolamine, a memory function suppressing drug (Nissen et al., 1987). Conversely, some authors have suggested that the learning modes are not mutually exclusive, but rather occur in parallel (Willingham & Goedert-Eschmann, 1993; Penhune & Steele, 2012). However, neuroimaging has not been able to substantiate this claim, with studies demonstrating explicit and implicit learning to have distinct neural representations, further supporting the independent systems theory (Doyon et al., 1996; Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998).

Additionally, motor sequence learning has been proposed to be comprised of spatial and motor components that dominate at different stages of the learning process (Dahms et al., 2019; Hikosoka et al., 1999; Hikosoka, Nakamura, Sakai, & Nakahara, 2002; Penhune & Steele, 2012). In early learning, performance changes are mediated by learning the spatial characteristics such as stimulus-response associations and spatial locations (Nakamura et al., 2002; Penhune & Steele, 2012). These characteristics are processed explicitly and acquired rapidly. Conversely, improvements in late learning are dominated by the motor components such as timing and speed (Dahms et al., 2019; Hikosoka et al., 2002). These characteristics are processed implicitly, acquired slowly, and necessitate minimal attentional resources. Neural investigations have also been conducted to determine the cortical contributions of each stage and component of motor sequence learning.

1.2. Neural correlates of motor sequence learning

A large number of neuroimaging studies have examined the contribution of cortical and subcortical regions to motor sequence learning. The current understanding is that motor sequence learning is supported by two distinct neural networks: cortico-cerebellar and cortico-striatal

(Hikosaka et al., 1999; Hikosaka et al., 2002; Lohse et al., 2014). The contribution of each neural network has been shown to differ depending on the stage of motor sequence learning. Early motor sequence learning has been shown to be predominately supported by the cortico-cerebellar network, while late motor sequence learning has been found to rely on the cortico-striatal network (Penhune & Doyon, 2002). However, it is important to note that there are some inconsistencies regarding stages of learning having distinct network contributions. These findings are outlined in the following paragraph.

Studies have shown the cerebellum to be active in the early stage but progressively decrease with learning (Doyon et al., 2002; Floyer-Lea & Matthews, 2004; Floyer-Lea & Matthews, 2005; Jenkins et al., 1994; Lehericy et al., 2005; Wu et al., 2004). The early activation is suggested to reflect plasticity at the granule-Purkinje cell synapses associated with the formation of internal models (Doyon et al., 2002; Penhune & Steele, 2012), while the decrease is suggested to represent the cerebellum's role in the task becoming automatic (Jenkins et al., 1994; Marr, 1969; Wu et al., 2004). No change in cerebellar activation has also been observed (Grafton et al., 1995); however, this was attributed to the limited view of the positron emission tomography (PET) scanner (Doyon et al., 2003; Jenkins et al., 1994). Additionally, increased contribution of the dentate nucleus has been reported in the later phase of learning (session 2 of 3), suggesting activation between the nucleus and cerebellar cortex may differ depending on the amount of practice (Doyon et al., 2002). Increased involvement of the parietal cortex, prefrontal cortex (refer to section 1.3), and premotor cortex has also been observed during early learning compared to late learning (Dahms et al., 2019; Jenkins et al., 1994; Jueptner et al., 1997a, b; Poldrack et al., 2005; Toni et al., 1998; Wu et al., 2004). The increase in parietal activity is suggested to reflect an increase in movement preparation (Jueptner et al., 1994; Toni et al., 1998;

Wu et al., 2004) and spatial attention during early learning (Jenkins et al., 1994). However, increased contributions from the parietal cortex have also been observed during the late phase of learning (Doyon et al., 2002; Eliassen et al., 2001; Grafton et al., 1995), when awareness of the sequence was achieved. This pattern of activation is suggested to reflect explicit knowledge. In terms of the premotor cortex, increased activation during early learning is suggested to reflect mechanism that are important for motor acquisition such as temporal organization, movement selection (Deiber et al., 1991), and generation of motor sequences from memory (Wu et al., 2004). Similar to the parietal cortex, learning-related increases in the premotor cortex have also been observed after achieving explicit sequential knowledge (Grafton et al., 1995; Hazeltine et al., 1997).

Learning-related decreases in the cerebellum, parietal cortex, PFC, and premotor cortex have also been accompanied by increased activations in the primary motor cortex (M1), supplementary motor area (SMA), and basal ganglia (Doyon, Penhune, & Ungerleider, 2003; Doyon et al., 2002; Grafton et al., 1995; Jenkins et al., 1994; Karni et al., 1995; Karni et al., 1998; Toni et al., 1998). Increased M1 activation following practice is postulated to reflect long-term representation of the motor sequences (Karni et al., 1998). However, studies have also revealed an absence of M1 activation (Doyon et al., 2002; Toni et al., 1998) and an increase in activation but only with implicit learning (Grafton et al., 1995). The former is thought to be associated to the level of practice, with activation only apparent if the task is overlearned (Doyon et al., 2002). Similar to Karni et al. (1998), increased activation of the SMA is suggested to reflect the representation of well-learned motor sequences (Doyon et al., 2002; Jenkins et al., 1994). It is also suggested to reflect when a task is over-learned and no longer needs an external cue for movement generation (Jenkins et al., 1994). Likewise, studies have suggested that

increased contributions from the basal ganglia reflect long-term motor sequence storage (Doyon et al., 2003), as well as the combination of several movements into chunks (Dahms et al., 2019; Penhune & Steele, 2012). However, some neuroimaging studies have observed activation of the striatum in early learning, suggesting it is related to mental rehearsal or executive function (Jenkins et al., 1994; Jueptner et al., 1997; Toni et al., 1998).

As alluded to earlier, studies have also shown explicit and implicit learning to exhibit differing neural representations. Implicit learning has been shown to activate regions such as M1, SMA, and basal ganglia (Doyon et al., 1996; Eliassen et al., 2001; Hazeltine et al., 1997; Honda et al., 1998; Penhune & Steele, 2012). Conversely, explicit learning has been found to activate prefrontal cortex, parietal cortex, and premotor regions (Doyon et al., 1996; Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998). In addition, several of the aforementioned studies (Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998) observed lateralized neural representations, with explicit learning activating the ipsilateral hemisphere and implicit learning activating the contralateral hemisphere.

Based on the several inconsistencies highlighted above, an alternative view on the neural contributions to motor sequence learning has been proposed (Doyon et al., 2003; Doyon & Benali, 2005). The model suggests that early learning requires contributions from both, the cortico-cerebellar and cortico-striatal networks (e.g., striatum, cerebellum, premotor, SMA, PFC, and parietal cortex). The involvement of both networks is important for establishing the motor routines necessary for learning the motor sequence task. As learning progresses and performance reaches the automatic phase, the skill is represented across only one network: cortico-striatal (e.g., striatum, SMA, M1 and parietal cortex). The network is then reactivated if the task is initiated after a prolonged period (e.g., retention). This proposal is partially in line with the

concept of spatial and motor components postulated by Hikosaka et al. (1999) (refer to section 1.1). According to Hikosaka et al. (1999), the task is acquired as a spatial sequence in early learning, with contributions from the association cortex, which includes PFC, parietal cortex, and the anterior basal ganglia. After practice, the task is acquired as a motor sequence with contribution from the SMA, M1, middle basal ganglia and the dentate nucleus. Clearly, further research is required to resolve the inconsistencies in neuronal findings to better understand their contribution to motor sequence learning.

1.3. Change in prefrontal cortex (PFC) activation during motor sequence learning

Similar to the cortical and subcortical structures discussed in section 1.2., heterogeneous findings have also been observed with the PFC. PFC activation has been reported to decrease (Jenkins et al., 1994; Jueptner et al., 1997b; Poldrack et al., 2005; Toni et al., 1998; Wu et al., 2004), as well as increase (Doyon et al., 1996; Doyon et al., 2002; Eliassen et al., 2001; Grafton et al., 1995; Hazeltine et al., 1997; Penhune & Doyon, 2002) during motor sequence learning.

Jenkins et al. (1994) investigated the neural correlates associated with learning an eight unit keypress sequence using PET. Participants were evaluated under three conditions: rest, while performing a pre-learned sequence (PRE), and while performing a new sequence (NEW). The pre-learned sequence was acquired 90 minutes prior to scanning. The results revealed increased activation in the right DLPFC and ventrolateral PFC (VLPFC) only during the NEW condition. Jenkins et al. (1994) suggested that the increased activation was associated with the trial and error paradigm that required participant to freely generate their sequential movements. With practice completed, participants no longer needed to adhere to the trial and error paradigm; they executed the sequence as learned. In addition, the authors attributed the extensive right

hemisphere activation to the task involving spatial processing. However, learning was assessed in a single session with no retention test to evaluate the relative permanence of the skill.

Building on the work of Jenkins et al. (1994), Jueptner et al. (1997b) used PET to examine the effect of attention on the functional anatomy of an eight unit keypress sequence acquired through trial and error. Participants were scanned under four conditions: pre-learned sequence (PRE), new sequence (NEW), attention directed towards the pre-learned sequence (ATT), and baseline (BASE). Similar to Jenkins et al. (1994), the DLPFC was activated bilaterally during new learning but not during the automatic condition (PRE). Interestingly, increased activation was observed in the left DLPFC in the ATT condition compared to the PRE condition. The authors concluded that increased activation in early learning reflects mental rehearsal of the sequential movements, whereas increased activation during the attentional direction condition reflects preparation of an upcoming motor response. A similar limitation to Jenkins et al. (1994) was the short acquisition period and no retention test.

Toni et al. (1998) examined the blood oxygen level-dependent (BOLD) signal of fMRI during the trial and error learning of an eight unit keypress sequence. Participants completed 45 active blocks (sequence) in an on-off cycle paradigm. By block nine, participants were able to perform the sequence with no errors. The neural results revealed increased activation of the right DLPFC during the early phase of learning, followed by a decrease in activation once the task was learned. Similar to Jueptner et al. (1997b), the authors concluded that the increased activation could reflect attention, movement preparation, or mental rehearsal. Unfortunately, the study did not attempt to distinguish between the aforementioned processes.

Wu et al. (2004) examined changes in cortical activity before and after automaticity of self-initiated sequential movements was achieved. Participants were instructed to perform two

sequences of finger-tapping, one four units in length and the other 12 units in length. Following the initial scan, participants were allotted two to four hours of practice, after which automaticity was evaluated using a dual-task paradigm (sequence + a visual letter-counting task). The findings revealed decreased activation in the left DLPFC following practice. Wu et al. (2004) concluded that reduced activation in the left DLPFC reflects less of a need for timing control once automaticity is achieved.

Poldrack et al. (2005) investigated the neural correlates of movement automaticity using the SRTT under single-task (ST) and dual-task (DT) conditions. In the SRTT, participants were presented with a visual target in one of four screen locations and required to respond as quickly as possible by pressing one of four spatially compatible buttons. The scanning sessions consisted of four conditions: ST sequence, DT sequence, ST pseudorandom, and DT pseudorandom. In the sequence blocks, a 12-item sequence was presented, unbeknownst to the participants. In the pseudorandom blocks, items were presented in a pseudorandom order to ensure differences between the two conditions reflected sequential knowledge. For the secondary task, participants were instructed to count the number of times a high tone was presented. Participants were scanned before and after undergoing three training sessions. Findings revealed reduced activation in the right VLPFC in the ST sequence condition and in the right DLPFC and bilateral VLPFC in the DT sequence condition. Poldrack et al. (2005) concluded that automaticity was associated with decreased activation in both control regions. In addition, the decrease in DLPFC activation observed in the DT condition was suggested to reflect a reduction in stimulus-response conflicts following the achievement of movement automaticity.

Conversely, numerous studies have reported increased activation of the PFC following motor sequence learning. Grafton et al. (1995) examined changes in activation associated with

explicit and implicit motor sequence learning. Explicit learning during the SRTT revealed increased contributions from the right DLPFC. The authors concluded that this activation reflects the involvement of spatial working memory in representing the stimulus and/or response locations. The authors also postulated that activation of the right DLPFC could reflect retrieval of episodic memory.

Doyon et al. (1996) investigated changes in neural activation during the early and late learning phases of a visuomotor sequence task. Similar to the SRTT, participants were presented with a visual target in one of four locations and required to respond as quickly as possible by pressing one of four spatially compatible buttons on the screen. The experiment was divided into a practice session that lasted 45 minutes and a PET scanning session comprised of six conditions: highly learned sequence, random sequence, newly learned sequence, perceptual control, declarative memory for the highly learned, and declarative memory for the newly learned. Similar to Grafton et al. (1995), the findings revealed increased activation in the right mid-ventrolateral frontal cortex once explicit knowledge of the highly learned sequence was acquired. Doyon et al. (1996) concluded that activation of the mid-ventrolateral frontal cortex in the late phase of learning reflects explicit retrieval of information from long-term memory.

Building on the work of Grafton et al. (1995), Hazeltine et al. (1997) investigated the neural correlates of explicit and implicit learning when responses are cued by colour rather than position. Similar to Grafton et al. (1995), activation of the right PFC was observed with explicit learning. However, explicit learning with colour cues activated more ventral regions of the PFC compared to spatial cues. For implicit learning, no significant difference was observed between colour and spatial cues. The authors concluded that the contribution of cortical regions including

the PFC are dependent on stimulus characteristics and awareness (explicit vs. implicit knowledge).

Similarly, Eliassen et al. (2001) examined changes in cortical activation during the production and encoding of a motor sequence. Participants were required to perform a version of the SRTT under four conditions: random sequence, secret sequence (implicit), learned sequence (explicit), and watch (passive). The findings were two-fold: increased activation of the PFC with explicit knowledge and increased activation of the PFC and inferior parietal lobule as learning progressed. Eliassen et al. (2001) suggested that PFC activation is necessary to facilitate the acquisition and working memory requirements (e.g., retrieval) associated with sequential learning.

Doyon et al. (2002) examined changes in cortical activation associated with the acquisition of an explicit motor sequence. Participants performed a version of the SRTT under three conditions: perceptual (no movement), random and learned. For the learned condition, participants were required to memorize the sequence to reduce any cognitive influence confounds. Participants were scanned across three sessions with 30 minute practice periods in between the sessions. Findings revealed increased activation in the right VLPFC. The authors suggested that activation of the VLPFC was associated with the demands of the task, specifically maintaining the sequential movements in their correct temporal order. This study was the first to examine motor sequence learning over a longer period of time (multiple sessions vs. single session).

Penhune and Doyon (2002) investigated the contributions of cortical and subcortical regions to the acquisition and retention of timed motor sequences. Unlike the aforementioned studies, the authors included a retention period, four weeks after acquisition to evaluate the

relative permanence of the motor sequence skill and its associated cortical activations.

Participants were instructed to reproduce a complex timed motor sequence in synchrony with a visual stimulus in two conditions: learned (LRN) and isochronous baseline (ISO). For the LRN condition, participants were presented with two sequences and two tempos. Participants were scanned on three separate days: day one of learning, after five days of practice and after a four week delay. The findings revealed increased activity in the VLPFC and orbital frontal lobe on day five. No activation of the PFC was observed during retention. The authors concluded that activation of the VLPFC following learning reflects memory retrieval, while activation of the orbitofrontal reflects intrinsic reward associated with improved performance.

The reviewed literature demonstrates that a decrease in activation of the PFC from early to late learning reflects primarily movement-related mechanisms such as movement preparation, stimulus-response conflicts, spatial processing, and automaticity. Conversely, with the basal ganglia and motor cortices involved in the long-term storage and representation of well-learned sequences, an increase in activation from early to late learning is predominately suggested to reflect working memory functions such as explicit learning and retrieval. However, it is important to point out that with varying practice scales, the majority of studies likely represent cortical changes associated with the fast learning stage and/or a transition into the slow learning stage (Doyon et al., 2009). Furthermore, with the high cost associated with PET and fMRI, it is difficult for studies to evaluate the relative permanence of motor sequence learning (Lohse et al., 2014).

1.4. Continuous wave functional near-infrared spectroscopy (fNIRS)

fNIRS is an indirect and non-invasive neuroimaging technique that monitors changes in cerebral oxygenation associated with the neurovascular coupling response (Perrey, 2008). During cortical activity, there is an increase in the metabolic demand for oxygen and glucose that is met by an increase in cerebral blood flow (CBF) and cerebral blood volume (CBV) (Bunce et al., 2006; Perrey, 2008; Pinti et al., 2008). This in turn, causes an increase in the oxyhemoglobin concentration ([HbO]) and a corresponding decrease in the deoxyhemoglobin concentration ([HbR]) (Pinti et al., 2018; Scholkmann et al., 2014). HbO and HbR, also known as chromophores, can then be measured with fNIRS by exploiting their optical properties.

Unlike most biological tissues (e.g., hair, scalp, skull, and cerebrospinal fluid), HbO and HbR have absorption properties in the 700 – 900 nanometer near-infrared range (Bunce et al., 2006; Scarapicchia, Brown, Mayo, & Gawryluk, 2017). Using this optical window, photons are propagated through the tissue, after which they are scattered and absorbed (Bunce et al., 2006; Perrey, 2008). The attenuated light, which travels through a banana-shaped trajectory, is then measured at the scalp. From there, the modified Beer-Lambert law, which considers attenuation in a scattering medium, is used to quantify the relative concentrations of HbO and HbR.

As such, fNIRS provides several advantages over classic neuroimaging techniques such as fMRI and PET (Bunce et al., 2006; Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). This includes low cost, portability, high temporal resolution, and higher tolerance to motion artifacts. Consequently, this enables long-term evaluation in more ecologically valid environments compared to fMRI and PET, which not only confines investigations to the magnetic bore and gantry, respectively, but is associated with a high implementation cost. These advantages have enabled the application of fNIRS in a variety of domains such as psychology (e.g., attention, emotion, executive function, etc.), linguistics, brain-computer interface, disease

(e.g., Alzheimer's disease, Parkinson's disease, stroke, etc.), and motor control (e.g., gait) (see Quaresima & Ferrari, 2019 for a review). Additionally, while fMRI relies on the paramagnetic properties of HbR, fNIRS can quantify the concentration changes of HbO and HbR (Herold, Wiegel, Scholkmann, & Muller, 2018). Furthermore, while both techniques measure the hemodynamic response, the sampling rate of fNIRS (e.g., up to 10 Hz) enables better tracking of the hemodynamic response function (HRF) by oversampling the data (Pinti et al., 2018).

However, as with any neuroimaging technique, fNIRS presents several disadvantages (Bunce et al., 2006; Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). This includes low spatial resolution, separation of signal contaminates, and lack of standardized signal processing practices (Cui, Bryant, Glover, & Reiss, 2011; Perrey, 2008; Pinti et al., 2018). With the penetration depth of the NIR light being proportional to the distance between the source and detector (e.g., 2 – 3 centimeters), only superficial cortical structures (e.g., PFC, M1, SMA, parietal cortex) can be evaluated (Perrey, 2008; Pinti et al., 2018). Additionally, hemodynamics arising from the extracerebral layer are difficult to isolate and can therefore, compromise the fNIRS signal (Tachtsidis & Scholkmann, 2016). This, combined with a lack of standardized signal pre-processing practices, can result in the misrepresentation of findings (Hocke et al., 2018).

1.5. Pre-processing of fNIRS

There are three sources of noise that can affect the quality of the fNIRS signal: instrumental noise (e.g., surrounding environment, electrical noise arising from the system, etc.), experimental error (e.g., motion artifacts), and physiological contaminants (e.g., cardiac output, respiration, Mayer waves, skin blood flow, etc.) (Huppert, Diamond, Franceschini, & Boas,

2009; Naseer & Hong, 2015; Yucel et al., 2021). If not properly addressed, the noise can significantly compromise the recovery of the HRF. To resolve this issue, numerous methods have been developed to improve the signal quality.

The first category of methods involves measuring noise independently from the fNIRS signal (Cooper et al., 2012; Cui, Bray, & Reiss, 2010). For example, head movement can be measured with an accelerometer or short-optode channels can be used to measure hemodynamics in the extracerebral layer. These auxiliary signals, comprised of noise, can then be subtracted from the fNIRS signal using adaptive filtering (Cooper et al., 2012; Cui et al., 2010; Scholkmann, Spichtig, Muehlemann, & Wolf, 2010; Zhang et al., 2007). However, the effectiveness of adaptive filtering is dependent on the external signal and the degree of correlation between the evoked cortical activity and the physiological contaminants (Scholkmann et al., 2010; Zhang et al., 2007).

The second category of methods can be applied directly to the fNIRS signal. These methods include but are not limited to principle component analysis (PCA) (Wilcox, Bortfield, Woods, Wruck, & Boas, 2005; Zhang, Brooks, Franceschini, & Boas, 2005), wavelet filtering (Molavi & Dumont, 2012), spline interpolation (Scholkmann et al., 2010), correlation-based signal improvement (CBSI) (Cui et al., 2010), and bandpass filtering. PCA is an eigenvector-based function that identifies signal components that account for the largest amount of spatial covariance and then removes those subsequent components (Wilcox et al., 2005; Zhang et al., 2005). It is a multi-channel approach that requires motion artifacts to be present in multiple channels, as well as operates under the assumption that motion artifacts are independent of the HRF (Brigadoi et al., 2014). However, these requirement are often violated by localized artifacts (e.g., movement of facial muscles) and/or temporally correlated motion artifacts (Brigadoi et al.,

2014). Wavelet filtering transforms the signal using the discrete wavelet transform, which isolates motion artifacts as abrupt changes in the frequency content of the signal (i.e., wavelet coefficients with the largest values) (Molavi & Dumont, 2012). While extremely effective at removing high-frequency spike artifacts, it can underestimate the HRF if motion artifacts are similar in frequency to the HRF (Brigadoi et al., 2014; Molavi & Dumont, 2012). Spline interpolation applies a cubic spline interpolation function to each identified motion artifact segment and then subtracts it from the original signal (Scholkmann et al., 2010). It is effective at removing baseline shift, however, requires motion artifacts to be identified prior to application. Therefore, if motion artifacts are difficult to identify (e.g., similar in amplitude and frequency to the HRF), the spline interpolation will not be properly applied and signal quality will not be improved (Brigadoi et al., 2014). CBSI is based on the assumption that HbO and HbR are negatively correlated and if motion artifacts are present in the signal, the correlation should become positive (Cui et al., 2010). This method is effective at removing motion artifacts of varying amplitude and improving the contrast-to-noise ratio (CNR). However, the underlying assumption is not always met, as an increase in CBF in response to cortical activation can precipitate a simultaneous increase in HbO and HbR (Fujiwara et al., 2014). Bandpass filtering is commonly used to remove instrument noise and certain physiological contaminants (Hocke et al., 2018; Huppert et al., 2009; Yucel et al., 2021). Low-pass filters can be used to remove high frequency instrument noise and cardiac oscillations (~ 1 Hz) (Kirilina et al., 2013). High-pass filters can be used to remove low-frequency drift (> 0.01 Hz) (Huppert et al., 2009). However, physiological noise related to respiration (~ 0.3 Hz) and blood pressure (i.e., Mayer waves; ~ 0.1 Hz) are generally advised not to be removed, since they can overlap with the frequency profile of the HRF (Huppert et al., 2009; Yucel et al., 2021).

However, the heterogeneity in processing methods and their accompanying strengths and weaknesses makes it challenging to select an appropriate processing approach. The issue is compounded further by poor reporting practices and a lack of standardization (Hocke et al., 2018; Pinti et al., 2018; Yucel et al., 2021). For example, Pinti et al. (2019) demonstrated that in a 110 papers, 36.3% failed to provide information on the type of bandpass filter (e.g., Butterworth, moving average, finite impulse response, etc.) used. The absence of information on filter type as well as processing pipelines makes it difficult for future studies to replicate protocols and findings. Additionally, several of the previously mentioned methods (e.g., PCA, wavelet, spline, and bandpass) require tuning parameters (i.e., user defined values) that have yet to be standardized. The improper selection of a tuning parameter can significantly impact the results and subsequent interpretations (Hocke et al., 2018; Pinti et al., 2019). Therefore, in an effort to provide processing guidelines, studies have compared the performance of several motion correction techniques (e.g., PCA, Kalman filtering, targeted PCA, wavelet filtering, spline interpolation, CBSI, and moving average) (Behrendt, Firk, Nelson, Perdue, 2018; Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al., 2015). Cooper et al., 2012 revealed that spline and wavelet produced the greatest improvement in mean-squared error (MSE) and CNR, respectively. Brigadoi et al., 2014 reported wavelet to be the best at reducing area under the curve. Similarly, Hu et al. 2015 found wavelet to the best approach at reducing high frequency spiked artifacts. Conversely, Di Lorenzo et al. 2019 observed that stacking spline and wavelet was the best for recovering the HRF and reducing the within- and between-subject standard deviation. Behrendt et al. (2018) found both, targeted PCA and wavelet to perform well in reducing the root-mean-squared error. However, the studies failed to make significant strides towards standardization, seeing as different tuning parameters were used for the wavelet function

(e.g., interquartile range values from 0.1 to 1.5) and different subsets of the population were evaluated (e.g., infants, stroke patients, and healthy adults).

1.6. Correlation between fMRI and fNIRS

To demonstrate the validity of fNIRS and support its continued application, a number of studies have also examined the correlation between fNIRS and the blood oxygen level dependent (BOLD) signal of fMRI (Huppert, Hoge, Diamond, Franceschini, & Boas, 2006; Kleinschmidt et al., 1996; Mehagnoul-Schipper et al., 2002; Sato et al., 2013). During a finger-tapping task, Huppert et al. (2006) reported a strong correlation ($r = 0.98$) between the BOLD response and HbR. Toronov et al. (2001) observed a comparable correlation, however, it was limited to 50% of the participants. The authors attributed the lack of consistency to signal contamination from the hemodynamic fluctuations in the extracerebral layer. To address this issue, Sato et al. (2013) examined not only the correlation between fNIRS and the grey matter BOLD signal but also the signals originating from the soft tissue and skin blood flow. Statistical analysis revealed that HbO and HbR were found to be correlated with the BOLD signal. Mehagnoul-Schipper et al. (2002) reported a similar correlation in sensorimotor activation in young and older adults. Collectively, this body of research demonstrates that the hemodynamic response derived from fNIRS is comparable to that of the BOLD signal measured by the gold standard fMRI.

1.7. Thesis outline and hypotheses

This dissertation outlines four experiments that were designed to investigate the capability and limitations of fNIRS in elucidating the role of the PFC in motor sequence learning and movement automaticity. The experiments involved sequential finger-tapping tasks that were

4 (Chapters 2 and 5), 8 (Chapter 3 and 4), and 12 (Chapters 2 and 5) units in length. With potential hemispheric differences in language processing, left-handed participants were excluded from all experiments. Right-handed participants were identified using the Edinburgh Handedness Inventory (Oldfield, 1971). In all experiments, fNIRS was acquired using an ON-OFF cycle paradigm of rest (baseline) and active (experimental condition). The rest block enabled all extraneous stimuli/factors to be accounted for, thereby allowing activation in the active block to be attributed to the executed task. The experiments involved either 5 active and 6 rest blocks (Chapters 2 and 5) or 3 active and 4 rest blocks (Chapters 3 and 4). For experiments 1 and 4 (Chapters 2 and 5), participants performed the tasks in a supine position to replicate similar fMRI conditions. For experiments 2 and 3 (Chapters 3 and 4), participants performed the tasks in a seated position.

Due to the constraints of a continuous-wave fNIRS (e.g., scattering and absorption properties), absolute concentration values could not be determined (Ferrari & Quaresima, 2012; Scholkmann et al., 2014). Therefore, the primary dependent variable for all experiments was the relative concentration change of oxyhemoglobin (HbO). Deoxyhemoglobin (HbR) was only included in Chapters 2 and 5 due to its poor sensitivity to motor-related hemodynamic changes (Holper et al., 2012; Miyai et al., 2001; Suzuki et al., 2004). The hemodynamic response function (HRF) was measured during the entire active block. Then, the HRF was averaged across blocks of the same condition to increase the signal-to-noise ratio and reliability of the fNIRS signal (Gervain et al., 2011). Additionally, motor performance was evaluated using measures such as error score (i.e., incorrect or missing key press) (Chapters 2, 3, and 5) and frequency of responding (Chapter 3).

As mentioned above, the overall objective of this dissertation was to elucidate the function of the PFC in motor sequence learning and movement automaticity using fNIRS. The objectives and hypothesis for each experiment are described below.

Experiment 1 (Chapter 2): Investigated the validity of fNIRS as a suitable technique to fMRI by replicating a previous motor sequence learning study (Wu et al., 2004). Specifically, we were interested to see if fNIRS could detect similar movement automaticity-related changes in the PFC. Two sequences of finger-tapping (4 and 12 units in length) were practiced over a 2 – 4 hour period. It was hypothesized that if movement automaticity was achieved, there would be a decreased activity in the left dorsolateral PFC (DLPFC), similar to Wu et al. (2004).

Experiment 2 (Chapter 3): Investigated changes in PFC activation following motor sequence learning. Building on the findings of Chapter 2, this experiment included 4 finger-tapping tasks (8 units in length), a control group, four practice sessions and a retention phase (24 hours after post-test). The retention phase was included to determine if the relative permanence (automaticity) of the motor tasks was achieved (Kantak & Winstein, 2012). It was hypothesized that if movement automaticity through learning was achieved, the trained group would exhibit decreased activity in the left hemisphere of the PFC between pre-test and retention, with no changes in activation observed in the control group. Additionally, it was hypothesized that learning the sequences explicitly would be represented by increased right hemisphere activation between pre-test and retention for the trained group only.

Experiment 3 (Chapter 4): Investigated how 5 motion correction techniques affect the statistical outcomes of a motor sequence learning study. The techniques include principal component analysis (PCA), wavelet filtering, spline interpolation, and correlation-based signal improvement (CBSI). They were selected according to previous literature (Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019) and their availability in the fNIRS processing program, HomER2 (Huppert et al., 2009). The second objective was to determine which motion correction technique would provide the most improvement in metrics such as recovered hemodynamic response function (HRF) and within-subject standard deviation. Given the methodological investigation, no hypotheses were put forward.

Experiment 4 (Chapter 5): Investigated changes in PFC activation associated with dual-task processing before and after motor sequence learning. Specifically, finger-tapping (4 and 12 units in length) was performed concurrently with a visual letter-counting task. Based on the increased activation of the right hemisphere observed in experiments 2 and 3, it was hypothesized that increased activation in the right dorsolateral PFC and ventrolateral PFC would be observed following explicit motor sequence learning. However, it is important to point out that this hypothesis contradicts albeit limited dual-task literature, which shows decreased activity in the dorsolateral PFC following motor sequence learning (Poldrack et al., 2005; Wu & Hallett, 2005).

1.8. References

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2. Chapter 2

A functional near-infrared spectroscopy (fNIRS) examination of how self-initiated sequential movements become automatic¹

¹ A version of this chapter has been published

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2.1. Abstract

The neural mechanisms underlying movement automaticity have been investigated using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) and, more recently, functional near-infrared spectroscopy (fNIRS). Since fNIRS is an emerging technique, the objective of the present study was to replicate the fMRI-related motor sequence findings as reported by Wu, Kansaku, and Hallett (2004) using fNIRS. Seventeen right-handed participants practiced self-initiated sequential finger movements of two lengths (4 and 12) until a level of automaticity was achieved. Automaticity was evaluated by performing a visual letter-counting task concurrently with the sequential finger movements. Our data was unable to replicate the pre- to post-practice decrease in cortical activity in the left dorsolateral prefrontal cortex for both motor sequence tasks. The findings did reveal increased contribution from the right hemisphere following learning. The observed lateralization is suggestive of explicit learning and the involvement of working memory in motor sequence production.

2.2. Introduction

A considerable body of literature exists on the neural correlates of movement automaticity (Grafton, Hazeltine, & Ivry, 1995; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Poldrack et al., 2005; Saling & Phillips, 2007; Toni, Krams, Turner, & Passingham, 1998; Wu & Hallett, 2005a, b; Wu, Kansaku, & Hallett, 2004). Functional imaging studies evaluating motor sequence learning have reported a decrease in cortical activation following learning in areas such as the premotor area, parietal cortex, dorsolateral prefrontal cortex (DLPFC), and cerebellum (Jenkins et al., 1994; Poldrack et al., 2005; Wu et al., 2004). It is suggested that as the movement becomes established, the movement draws on the same pre-learning neural regions but in a more efficient and economical manner (Saling & Phillips, 2007). Numerous studies have concluded this to be a sign of automatic movement processing (Poldrack et al., 2005; Wu et al., 2005a, b; Wu et al., 2004). This is based on the premise that automatic movements can be successfully performed with minimal demand for attentional resources (Bernstein, 1967; Fitts, 1964). Of interest, Wu et al. (2004) examined changes in cortical activation associated with the production of automatic sequential right hand finger movements. The movement was deemed automatic once the concurrent secondary task (e.g., visual letter-counting) was performed with minimal to no interference. Their findings revealed that learning precipitated decreased activation in structures such as the bilateral cerebellum, bilateral premotor area, posterior parietal cortex, pre-supplementary motor area (SMA), anterior cingulate cortex, left caudate nucleus, and left DLPFC. The authors concluded that most motor-related structures contribute to performing automatic movements but that their involvement becomes more efficient once learning is achieved.

However, it is important to point out that motor sequence learning has not consistently generated the same pattern of results (Saling & Phillips, 2007). In particular, studies have also reported learning to be accompanied by increased activation in regions such as supplementary motor area, motor cortex, and basal ganglia (e.g., putamen and globus pallidus) (Grafton et al., 1995; Jenkins et al., 1994; Poldrack et al., 2005; Seitz & Roland, 1992). One explanation is that the motor sequences are represented across both cortical and subcortical regions, but with the subcortical structures ultimately responsible for employing the established motor routines (Grafton et al., 1995; Saling & Phillips, 2007; Seitz & Roland, 1992). Collectively, the literature suggests two possible neural representations for movement automaticity. The first is an increased connectivity and efficiency of the neural network, as evidenced by a decrease in cortical activation (Saling & Phillips, 2007). The second is a reorganization of the functional anatomy from cortical to subcortical structures following learning (Grafton et al., 1995; Saling & Phillips, 2007; Seitz & Roland, 1992). For the purpose of this study, we will be focusing on the findings (Wu et al., 2004) that suggest automaticity is reflected by a decrease in activation.

It is important to point out that due to the structural limitations of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), all aforementioned studies investigated changes in cortical activation after learning while in a supine position. However, in the context of everyday motor activities, motor skill learning typically occurs in a seated or upright position (Hatakenaka, Miyai, Mihara, Sakoda, & Kubota, 2007). One neuroimaging method that can be applied in all positions and contexts is functional near-infrared spectroscopy (fNIRS). Similar to fMRI, fNIRS can provide an indirect measure of the hemodynamic response to cortical activity but by relying on the optical properties of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) (Leff et al., 2011; Perrey, 2008; Quaresima

& Ferrari, 2019; Scarapicchia, Brow, Mayo, & Gawryluk, 2017). With absorption in the near-infrared range of 700-900 nanometers (nm), fNIRS can quantify the relative concentrations of HbO and HbR by emitting light into the cerebral cortex and measuring the attenuation (Bunce, Izzetoglu, Izzetoglu, Onaral, & Pourrezaei, 2006; Perrey, 2008). However, with infrared light rapidly diffusing upon entry into the neuronal tissue, cortical structures of substantial depth cannot be examined (Perrey, 2008). For that reason, many studies using fNIRS target the prefrontal cortex (PFC) (Mihara & Miyai, 2016). While a significant limitation, fNIRS does have a number of advantages over the gold-standard fMRI. The most notable, is its portability (Scarapicchia et al., 2017). Many of the commercially available devices are small in size, Bluetooth-enabled, and battery-operated (Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). This enables the study of cortical function in more ecologically valid environments and as previously mentioned of tasks (e.g., standing, walking, face-to-face neuropsychological evaluations, etc.) not compatible with fMRI (Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). Additionally, fNIRS is more tolerant to motion artifacts as opposed to fMRI, which requires complete immobility to ensure valid data acquisition (Bunce et al., 2006; Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). This makes it a suitable method for examining populations that have difficulty remaining still (e.g., infants, children, and neurologically impaired individuals). Additionally, fNIRS provides a higher temporal resolution (approximately 1-10 milliseconds (ms)) than fMRI (Jasdzewski et al., 2003; Pinti et al., 2018), which allows for better identification of signal contaminants (e.g., physiological noise, motion artifacts) and hemodynamic onset (Perrey, 2008). Finally, its low cost makes it a more accessible neuroimaging tool and its ability to operate without causing interference makes it suitable for multimodal imaging (e.g., fNIRS-fMRI, fNIRS-EEG, and fNIRS-PET) (Pinti et al., 2018;

Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). However, fNIRS is still an emerging neuroimaging technique that requires further validation to ensure it can produce comparable findings to that of fMRI. Therefore, the objective of the present study was to replicate the motor sequence learning fMRI findings of Wu et al. (2004) in a supine position with fNIRS.

2.3. Materials and methods

2.3.1. Participants

Seventeen young adults participated in the study (23.25 ± 2.21 years). The participants were all right-handed as measured by the Edinburgh Handedness Inventory (Oldfield, 1971). All participants provided written informed consent, which was approved by the University of Ottawa Ethics committee. Participants were excluded if they had any self-reported neurological and/or visual impairments that could impact their performance.

2.3.2. Functional near-infrared spectroscopy (fNIRS)

Cortical activation was assessed through task-related changes in HbO and HbR using a multichannel, continuous-wave spectrometer (Artinis Medical Systems, Einsteinweg, Netherlands). The data was collected at a sampling rate of 10 Hz. The Octamon device consisted of eight light emitting diodes (760 and 850 nm) and two photodiode detectors, resulting in an 8-channel system (channels T1-T8). The optodes were configured on a headband so that channels T1-T4 were situated over the right hemisphere and channels T5-T8 over the left hemisphere. The optode distance was fixed at 35 mm. The optodes were situated on to the forehead using the 10/20 positioning system (Niedermeyer & Lopes de Silva, 2004) to ensure proper prefrontal cortex (PFC) placement. Although the optodes were not coregistered to an MNI brain template, it was presumed that based on their placement, T1/T7 were located over the dorsolateral PFC

(DLPFC), T2/T8 over the ventrolateral PFC (VLPFC), T3/T5 over the medial PFC (mPFC) and T4/T6 over the orbitofrontal cortex. To replicate the fMRI conditions reported in Wu et al. (2004), participants performed all tasks in a supine position with restricted movement, with the exception of the fingers of the right hand.

2.3.3. Additional apparatus

A four-button response box was used to record participants' sequence production. To keep track of the behavioural data, all key presses were transmitted to a computer equipped with National Instrument data acquisition software. A 13" monitor secured above the participant's head was used to provide visual signals.

2.3.4. Tasks

Following the protocol of Wu et al. (2004), participants were instructed to perform two sequences of right hand finger-tapping. The sequences were classified based on the number of presses (i.e., sequence-4 (S4) and sequence-12 (S12)). S4 consisted of 1-3-4-2, in which, 1, 2, 3, and 4 correspond to the index, middle, ring, and pinky fingers, respectively. S12 consisted of 1-4-3-2-2-4-1-3-4-1-2-3. To control for speed, both sequences were performed at 0.5 ± 0.1 Hz. To evaluate if automaticity was achieved, participants were asked to perform a visual letter-counting task concurrently with the sequential movements. The visual letter-counting task consisted of a random sequence of A, G, L, and O, and participants were required to count the total number of times the target letter (e.g., A) was presented. As set out by Wu et al. (2005), all movements were self-initiated, and the speed was not driven by an external cue.

2.3.5. Acquisition

fNIRS was acquired before (pre-test) and after (post-test) a level of automaticity was achieved. Prior to the pre-test acquisition, participants were given a brief period of time (approximately 10-20 minutes) to consistently replicate the required speed for both sequences. The acquisition sessions consisted of three conditions (S4, S12, and visual letter-counting) in an ON-OFF cycle paradigm of rest and active. For each condition, rest and active were 25-seconds in duration and were repeated six and five times, respectively. In the rest block, participants were instructed to relax and focus on the fixation cross displayed on the screen above them. In the active block, participants were instructed to perform S4, S12 or the visual letter-counting task. During the acquisition period, participants received no feedback with regards to their performance. After the pre-test, participants were allotted two to four hours of practice time. Prior to completing each sequence from memory 10 times in a row without error, participants completed at least two sets of 12 trials of 25-seconds in duration. After each set, participants received summary knowledge of results, which contained the number of trials with errors and the number of trials that exceeded the required speed. If an error was committed during the set of 10 trials, participants were required to perform another two sets of 12 trials. Before participants could be considered for the post-test, the sequences needed to be accurately performed while concurrently performing the visual letter-counting task. If an error was committed during this stage of practice, participants were required to start at the beginning by completing at least two sets of 12 trials, followed by the 10 trials in a row with no error.

2.3.6. Behavioural data

As previously mentioned, all key presses were recorded. A wrong key press was characterized as an error. Providing an incorrect total for the visual letter-counting task was classified as an error.

2.3.7. fNIRS data analysis

Data processing and analysis was performed with HomER (version 2), a MATLAB-based graphical user interface program designed for processing functional cortical data acquired from NIRS (Huppert, Diamond, Franceschini, & Boas, 2009). Following the conversion of data from raw intensity to optical density, each channel was inspected for motion artifacts. Specifically, if the signal for any given channel exceeded an optical density of 0.2 (AMPThresh) over 0.1 seconds (tMotion), it was masked for 0.2 seconds (tMask) before and after the artifact. Subsequently, a fourth-order Butterworth bandpass filter was applied to remove any additional sources of noise. A low-pass filter of 0.5 Hz was applied to remove high-frequency instrument noise and cardiac oscillations (Huppert et al., 2009). A high-pass filter of 0.01 Hz was applied to remove any low-frequency drift (Cooper et al. 2012). Following the bandpass, a principle component analysis (PCA) was applied to remove any remaining motion artifacts. The data was then converted from optical density to relative concentration using the modified Beer-Lambert law (Villringer & Chance, 1997). Lastly, a block average over a time range of -15-seconds and 25-seconds was calculated for each condition. The baseline of the average was set to zero by subtracting the mean of $t < 0$ from $t > 0$.

2.3.8. Statistical analysis

For each condition, the relative concentrations of HbO and HbR were analyzed using a channel (T1/T7, T2/T8, T3/T5, T4/T6) x session (pre, post) x hemisphere (right, left) repeated measures analysis of variance (ANOVA). When required, Least Significant Difference (LSD) was used to determine the location of significance. Statistical significance was set at $p < 0.05$. Behavioural data was analyzed using the Wilcoxon Signed-Rank Test.

2.4. Results

2.4.1. Behavioural data

All mean behavioural error data can be found in Table 2.1. Findings revealed a significant improvement in performance after practice for S4 ($Z = -2.414, p < 0.05$) and S12 ($Z = -3.063, p < 0.01$). Findings revealed significant improvements to performance after practice for both, S4 and S12 dual-tasks ($Z = -2.535, p < 0.05$ and $Z = -3.186, p < 0.01$, respectively).

Table 2.1

Performance (number of errors) of sequential finger movements and visual letter-counting before and after practice

Task	Errors	
	Before Practice	After Practice
Sequence-4	0.29 ± 0.63	0.02 ± 0.10
Sequence-12	1.45 ± 1.81	0.02 ± 0.10
Visual letter-counting	0 ± 0	0 ± 0
Sequence-4 and letter-counting	0.66 ± 1.18	0.01 ± 0.05
Sequence-12 and letter-counting	1.67 ± 1.82	0.29 ± 0.55

Note. Values are mean $\pm$ SD for the number of errors committed across the five active blocks.

For the sequences, errors were classified as incorrect finger movements. For the letter counting task, errors were classified as the difference between the correct total and the total provided by the participant.

2.4.2. Sequence-4 (S4)

2.4.2.1. Relative concentration change of oxyhemoglobin (HbO)

The main effects for channel, $F(3, 48) = 8.18, p < 0.01, \eta^2 = 0.338$, and hemisphere, $F(1, 16) = 6.89, p < 0.05, \eta^2 = 0.301$, were superseded by a channel x hemisphere interaction, $F(3, 48) = 3.99, p < 0.05, \eta^2 = 0.200$. The post-hoc analysis revealed: 1) right DLPFC (T1) exhibited significantly greater cortical activation than the left DLPFC (T7) ($p < 0.01$); and 2) left VLPFC (T8) was significantly less active than the right VLPFC (T2) ($p < 0.01$) (Figure 2.1 A). The main effect for session, $F(1, 16) = 0.15, p > 0.05, \eta^2 = 0.700$, was not statistically significant. All remaining interactions also failed to reach statistical significance ($p_s > 0.05$).

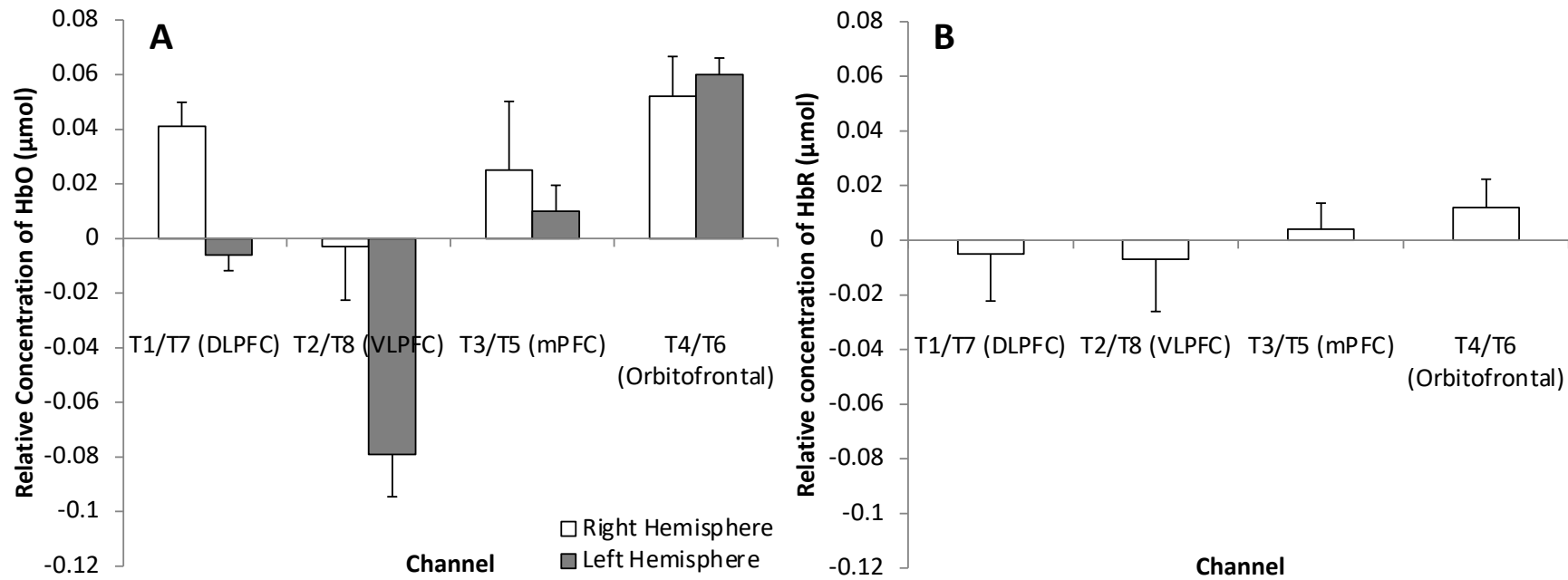


Figure 2.1. Change in relative concentration of HbO and HbR (mean $\pm$ SD) for S4. **(A)** Concentration differences in HbO as a function of channel and hemisphere. **(B)** Concentration differences in HbR as a function of channel.

2.4.2.2. Relative concentration change of deoxyhemoglobin (HbR)

The main effects for session, $F(1, 16) = 0.024, p > 0.05, \eta^2 = 0.002$, and hemisphere, $F(1, 16) = 16.71, p < 0.01, \eta^2 = 0.511$, were superseded by a session x hemisphere interaction, $F(1, 16) = 5.72, p < 0.05, \eta^2 = 0.263$. The post-hoc analysis revealed that the right hemisphere was significantly more active than the left hemisphere in the pre-test compared to the post-test ($p < 0.01$) (Figure 2.2). The main effect of channel, $F(3, 48) = 3.49, p < 0.05, \eta_p^2 = 0.179$, was statistically significant. The post-hoc analysis revealed that the cortical areas covered by channel pairs T1 and T7 (right and left DLPFC, respectively) and T2 and T8 (right and left VLPFC, respectively) were significantly more active than the areas covered by channel pair T4 and T6 (right and left orbitofrontal cortex, respectively) ($p_s < 0.05$) (Figure 2.1 B). All remaining interactions failed to reach statistical significance ($p_s > 0.05$).

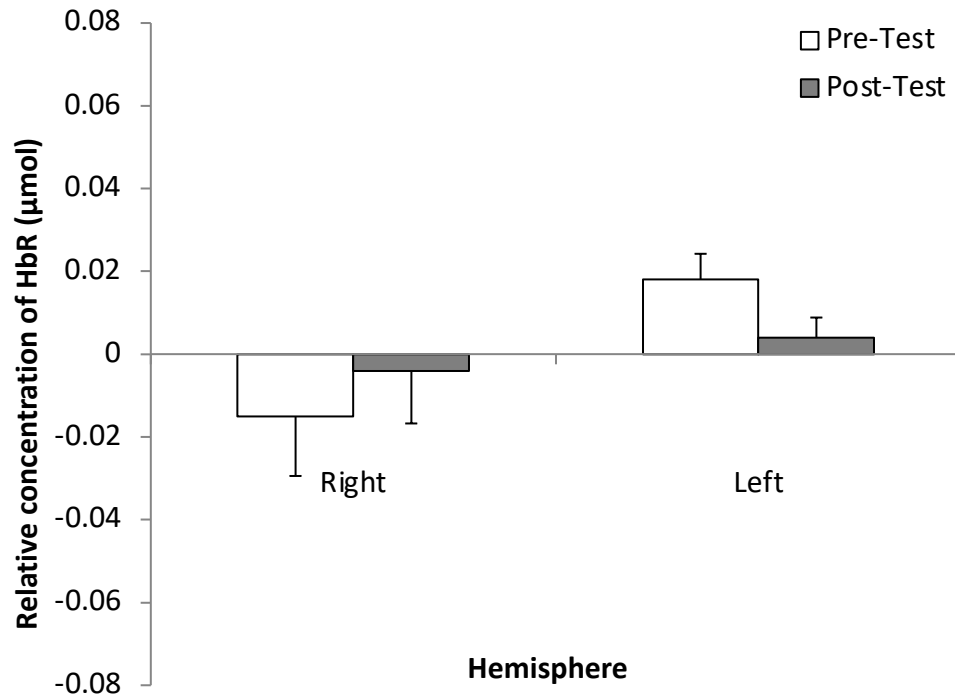


Figure 2.2. Change in relative concentration of HbR (mean $\pm$ SD) as a function of hemisphere and session for S4.

2.4.3. Sequence-12 (S12)

2.4.3.1. Relative concentration change of HbO

The ANOVA revealed a marginal trend for the session $\times$ hemisphere interaction, $F(1, 16) = 3.64$, $p = 0.074$, $\eta^2 = 0.186$. It appears that in the post-test, the right hemisphere displays greater cortical activation than the left, whereas in the pre-test both hemispheres exhibit minimal activation (Figure 2.3 A). The main effect of channel also revealed a marginal trend, $F(3, 48) = 3.03$, $p = 0.077$, $\eta^2 = 0.159$. This result suggests that cortical areas covered by channel pairs T1 and T7 (right and left DLPFC) and channel pairs T4 and T6 (right and left orbitofrontal cortex) were more active than cortical areas covered by channel pair T2 and T8 (right and left VLPFC)

(Figure 2.4). The main effect of session, $F(1, 16) = 1.28, p > 0.05, \eta^2 = 0.074$, and hemisphere, $F(1, 16) = 0.49, p > 0.05, \eta^2 = 0.029$, were not statistically significant. All remaining comparisons also failed to reach statistical significance ($p_s > 0.05$).

2.4.3.2. Relative concentration change of HbR

The ANOVA revealed a significant main effect of hemisphere, $F(1, 16) = 12.73, p < 0.01, \eta^2 = 0.443$, suggesting that the right hemisphere was significantly more active than the left hemisphere (Figure 2.3 B). The main effect of channel, $F(3, 48) = 4.69, p < 0.01, \eta^2 = 0.227$, was also statistically significant. The post-hoc analysis revealed that cortical areas covered by channel pairs T1 and T7 (right and left DLPFC, respectively) and T2 and T8 (right and left VLPFC, respectively) exhibited greater activity than the cortical areas covered by channel pairs T3 and T5 (right and left mPFC, respectively) and T4 and T6 (right and left orbitofrontal cortex, respectively) ($p_s < 0.05$) (Figure 2.4). The main effect of session, $F(1, 16) = 0.08, p > 0.05, \eta^2 = 0.005$, was not statistically significant. All interactions also failed to reach statistical significance ($p_s > 0.05$).

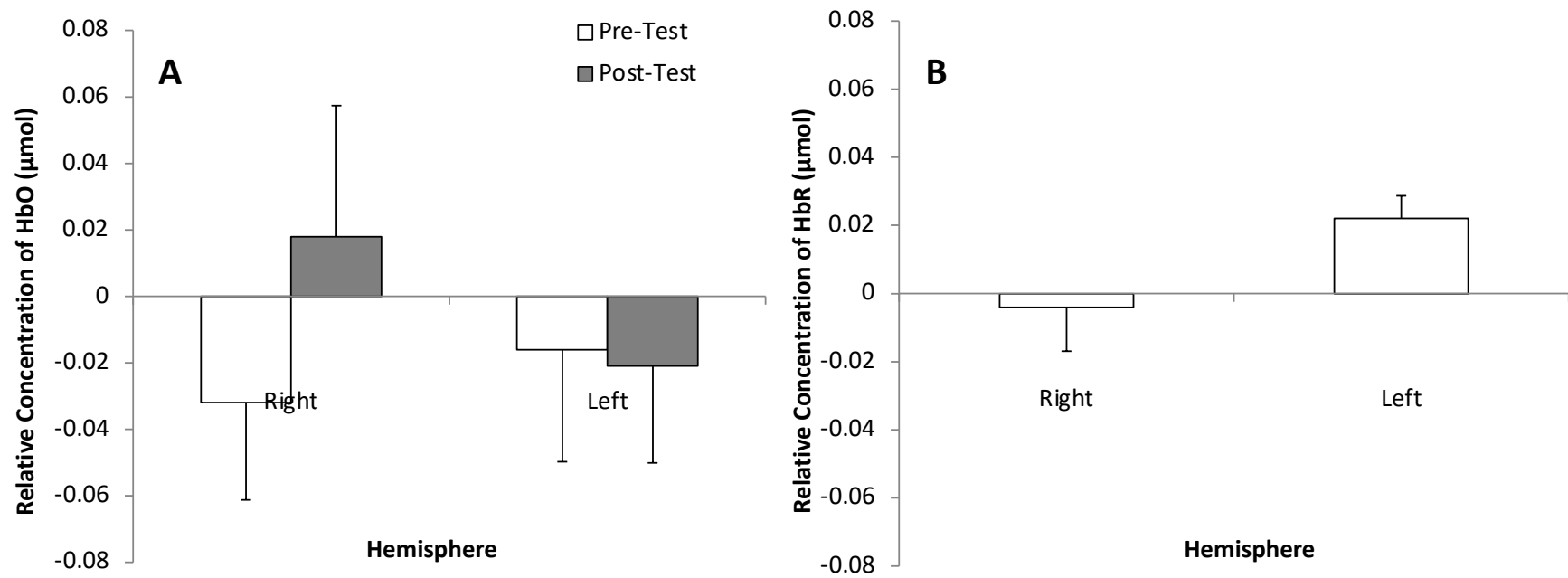


Figure 2.3. Change in relative concentration of HbO and HbR (mean $\pm$ SD) for S12. **(A)** Concentration differences in HbO as a function of hemisphere and session. **(B)** Concentration differences in HbR as a function of hemisphere.

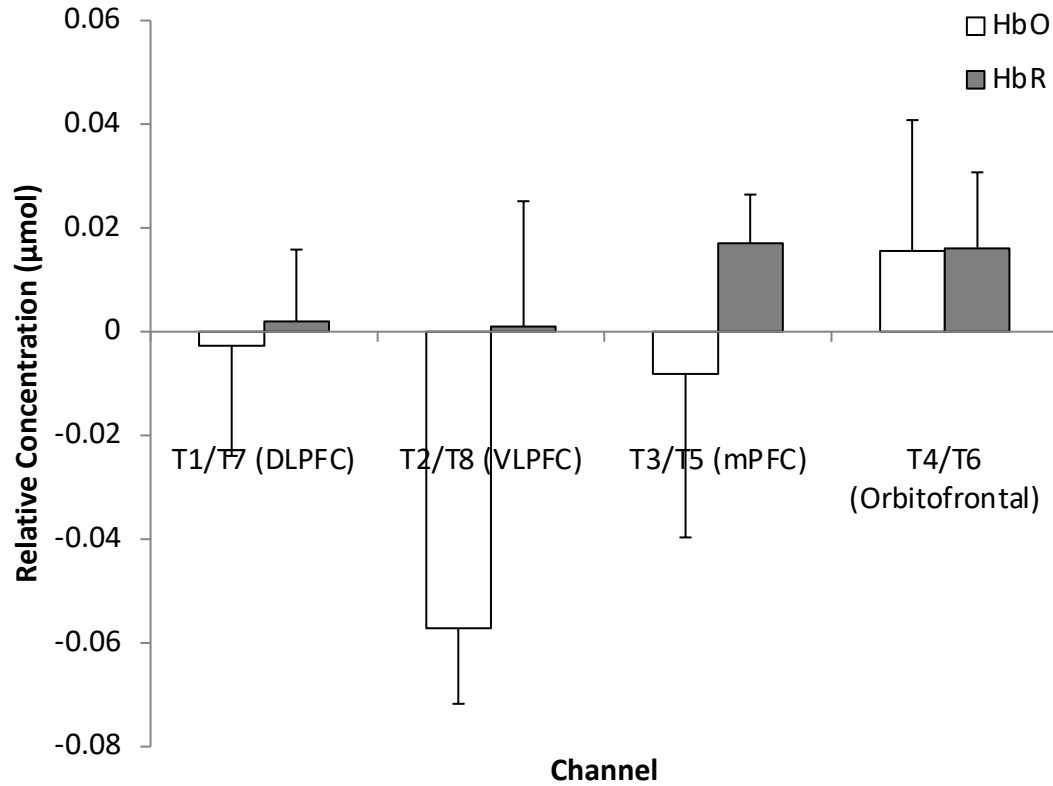


Figure 2.4. Change in relative concentration of HbO and HbR (mean $\pm$ SD) for S12 as a function of channel.

2.4.4. Visual letter-counting task

2.4.4.1. Relative concentration change of HbO

The ANOVA revealed a significant main effect of channel, $F(3, 48) = 3.52, p < 0.05$, $\eta^2 = 0.180$. The post-hoc analysis revealed: 1) cortical areas covered by channel pair T2 and T8 (right and left VLPFC, respectively) were significantly less active than cortical areas covered by channel pair T1 and T7 (right and left DLPFC, respectively) ($p < 0.05$); and 2) the cortical areas covered by channel pair T4 and T6 (right and left orbitofrontal cortex, respectively) were significantly more active than cortical areas covered by channel pair T3 and T5 (right and left mPFC, respectively) ($p < 0.05$). The main effects for session, $F(1, 16) = 1.02, p > 0.05, \eta^2 =$

0.060, and hemisphere, $F(1, 16) = 0.34, p > 0.05, \eta^2 = 0.021$, were not statistically significant. All interactions also failed to reach statistical significance ($p_s > 0.05$).

2.4.4.2. Relative concentration change of HbR

The ANOVA revealed a significant main effect of channel, $F(3, 48) = 3.64, p < 0.05, \eta^2 = 0.185$. The post-hoc analysis revealed that cortical areas covered by channel pairs T3 and T5 (right and left mPFC, respectively) and T4 and T6 (right and left orbitofrontal cortex, respectively) were significantly less active than areas covered by channel pair T2 and T8 (right and left VLPFC, respectively) ($p < 0.05$). The main effects for session, $F(1, 16) = 0.19, p > 0.05, \eta^2 = 0.012$, and hemisphere, $F(1, 16) = 0.25, p > 0.05, \eta^2 = 0.015$, were not statistically significant. All interactions also failed to reach statistical significance ($p_s > 0.05$).

2.5. Discussion

In this experiment, we aimed to use fNIRS to replicate the PFC-related changes in cortical activation, following the acquisition of automaticity as reported by Wu et al. (2004). Specifically, Wu et al. (2004) observed a decrease in cortical activity in the left DLPFC post-training for both motor sequence tasks (S4 and S12). The present data was unable to replicate the same pre to post-practice trend in cortical activity. We suggest that the practice period did not provide adequate time for the participants to reach automaticity, although improvements to motor performance were observed (Lang & Bastian, 2002). It is possible that the motor tasks require more practice time to ensure the actions are established and neural connectivity is enhanced (Saling & Phillips, 2007). For a bimanual coordination task, Debaere et al. (2004) reported a decrease in activation in the right DLPFC after participants underwent two days of

practice. Similarly, Poldrack et al. (2005) observed a decrease in the DLPFC activity following three sessions of practice separated by one or two days. Possibly, the post-practice decrease in the left prefrontal cortex would have been more pronounced and statistically significant if a prolonged practice period across multiple days was implemented. Although the movements appeared to be resistant to interference during the dual-task portion of practice (Doyon, Ungerleider, Squire & Schacter, 2002b), we suggest that with time, interference would emerge. In addition, this data suggests that task improvements may not be a guaranteed indicator of automaticity (Wu, Hallett, & Chan, 2015).

Due to the unexpected prevalence of right hemisphere-related findings, we do want to preface that the interpretations described below are theoretical but based on existing motor learning literature. Although marginal, the HbO analysis revealed that for S12, the right hemisphere exhibited higher activation during the post-test when compared to the pre-test. This is demonstrated in Figures 2.5 and 2.6, which provides a complete representation of the hemodynamic response associated with S12 before and after practice. HbR revealed corresponding findings with greater activity in the right hemisphere than the left, as evidenced by a decrease in HbR concentration. This finding parallels that of Grafton et al. (1995), who reported increased activation in the right DLPFC during the learning of a serial reaction time task. The authors postulated the increase to be associated with either the involvement of spatial working memory or the retrieval of verbal episodic memory. Along the same line, the present lateralization is thought to be associated with working memory and its role in the short-term storage of information retrieved from long-term memory (Magill, 2011; Petrides, 1994). It is well documented in the literature that the hemispheres of the prefrontal cortex have differential working memory functions (Tulving, 2002). Specifically, the left hemisphere is tasked with

encoding information while the right hemisphere is engaged during episodic retrieval (Fletcher, Shallice, Frith, Frackowiak, & Dolan, 1998; Kapur, Craik, Tulving, & Houle, 1997; Tulving, 2002). Therefore, it is possible that the increase in right hemisphere activation from pre- to post-test represents episodic memory retrieval (Magill, 2011; Tulving, 2002).

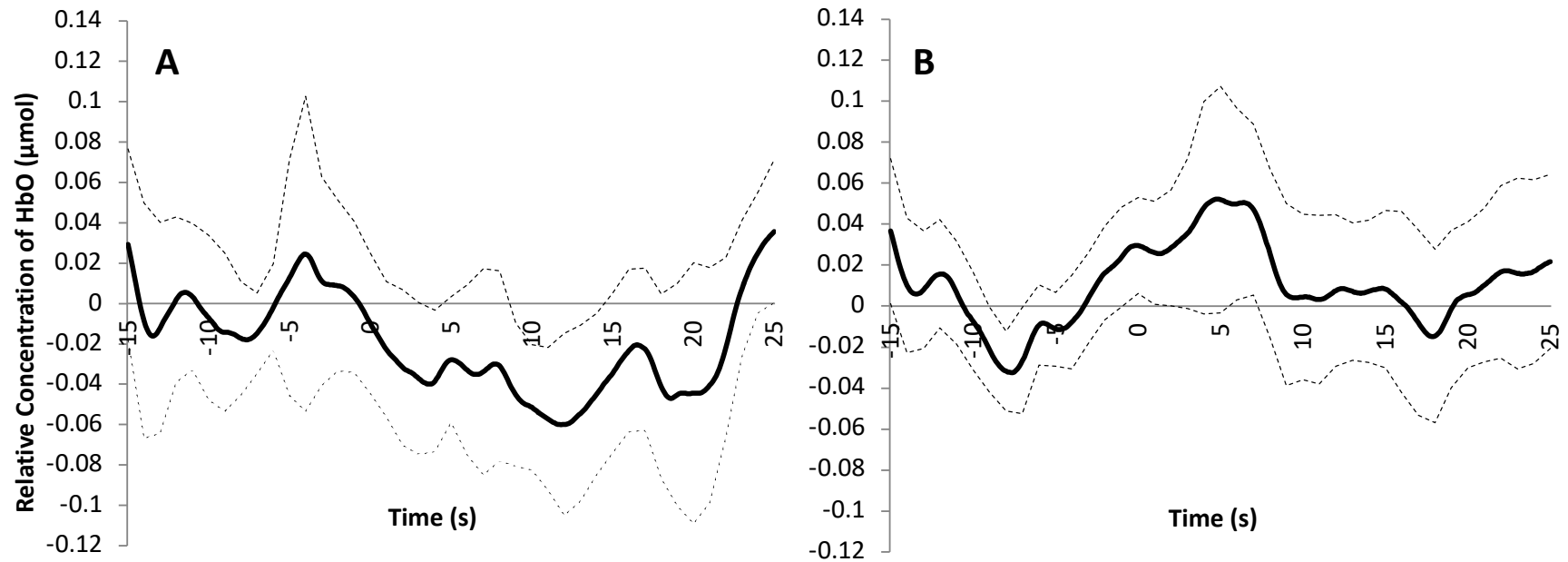


Figure 2.5. Hemodynamic time-course for the right hemisphere in response to S12 execution. **(A)** Relative concentration of HbO before and **(B)** after practice. The upper and lower dashed lines represent the upper and lower bounds, respectively of the 95% confidence interval. The active condition is 25-seconds and is preceded by a 15-second baseline.

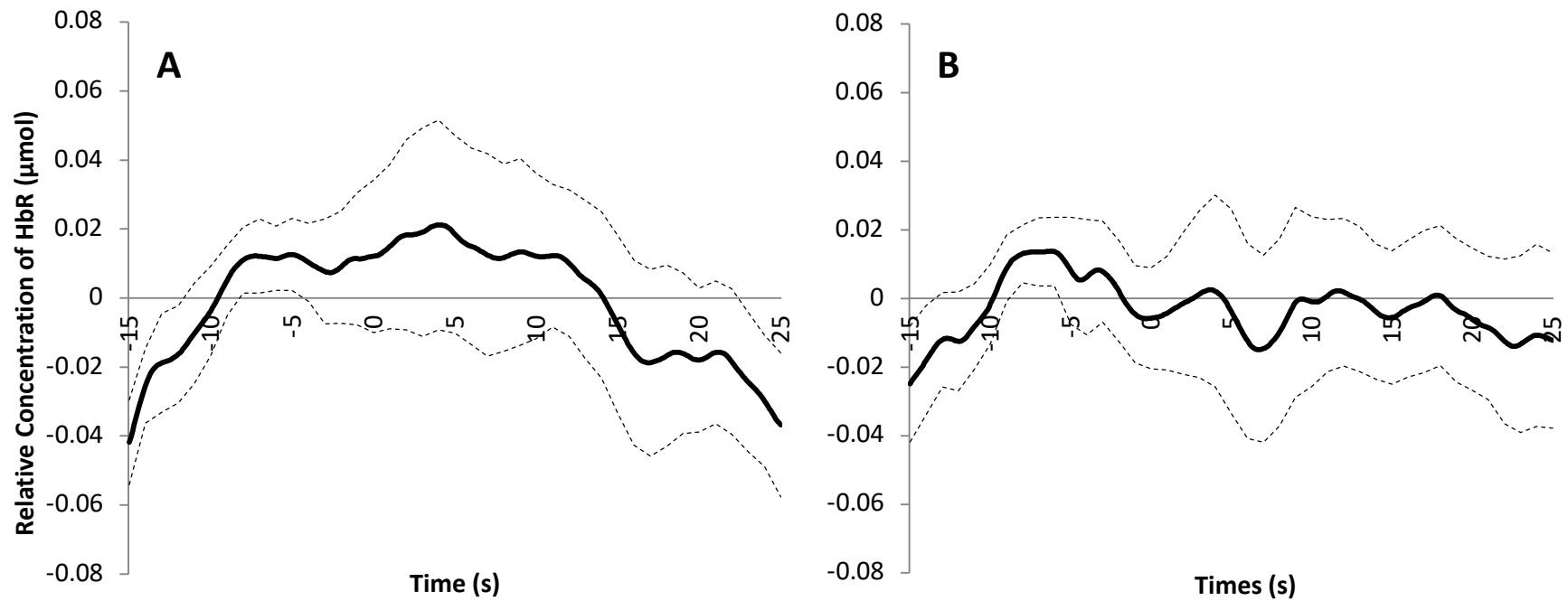


Figure 2.6. Hemodynamic time-course for the right hemisphere in response to S12 execution. **(A)** Relative concentration of HbR before and **(B)** after practice. The upper and lower dashed lines represent the upper and lower bounds, respectively of the 95% confidence interval. The active condition is 25-seconds and is preceded by a 15-second baseline.

Alternatively, learning the sequences explicitly may have contributed to the ipsilateral increase (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Grafton et al., 1995; Hazeltine, Grafton, & Ivry, 1997; Penhune & Doyon, 2002). This is consistent with several studies that have reported lateralized effects for explicit and implicit learning. Hazeltine et al. (1997) observed implicit learning of a motor sequence to be primarily localized to the motor and supplementary motor cortex of the contralateral hemisphere, whereas explicit learning was localized to the ipsilateral hemisphere in regions such as prefrontal cortex, premotor cortex, and temporal lobe. Similarly, Doyon et al. (1996) reported increased cerebral blood flow activity in the right mid-ventrolateral frontal area once explicit knowledge of the sequence was acquired. Therefore, the present results may suggest that the retrieval of a motor sequence necessitates the involvement of the right hemisphere, when acquired with explicit knowledge. This further supports the notion that motor sequence learning has lateralized neural representations (Hazeltine et al., 1997). In terms of the HbR results, no complimentary inferences can be made as no significant difference was found between the sessions and hemispheres.

In contrast to S12 findings, our HbR findings revealed greater right hemisphere activation than the left in the pre-test compared to the post-test for S4. It has been proposed that the frontal lobe is also critical for the exploratory processing of novel cognitive situations (Goldberg, Podell, & Lovell, 1994; Van Horn et al., 1998). Therefore, the observed lateralization prior to learning may be associated with the novelty of the task.

In addition, our findings revealed involvement from the DLPFC and VLPFC for both, S4 and S12. In particular, higher activation was observed in the right DLPFC and VLPFC when compared to the left hemisphere for S4. HbR revealed similar findings, with increased

contributions for the DLPFC and VLPFC compared to the orbitofrontal cortex. Comparably, for S12 production, increased activation ([HbR]) was observed in the DLPFC and VLPFC when compared to the orbitofrontal cortex and mPFC. There are a few possible explanations for the aforementioned results. The activation observed in the VLPFC regions may be related to the specific demands of the task (Doyon et al., 2002a). Doyon et al. (2002a) suggest that the VLPFC is engaged when maintaining the temporal order of a sequence of movements is required. Therefore, the contribution from the VLPFC may be associated with having to maintain a speed of 0.5 Hz for each sequence. Alternatively, the VLPFC has also been suggested to be critically involved in the explicit retrieval of motor sequences from long-term memory (Doyon et al., 1996; Petrides, 1994). The lateralization associated with explicit learning mentioned above (Doyon et al., 1996; Grafton et al., 1995; Hazeltine et al., 1997; Penhune & Doyon, 2002) and the activation of right VLPFC during S4 production may suggest that the observed activity is explicit memory related. The involvement of the DLPFC may also be related to the working memory requirements associated with the tasks. In several functional studies, the mid-DLPFC has been found to be involved when tasks necessitated monitoring of self-generated or externally generated sequence of responses (Petrides, 1996a, b). Therefore, having to actively monitor earlier finger-taps to ensure the upcoming responses were correct may explain the observed DLPFC involvement.

Similar to Wu et al. (2004), no significant difference was found between the pre- and post-test for the visual letter-counting task. The authors reported activation in several regions (e.g., SMA, parietal cortex, superior cortex, visual cortex, and cerebellum) but no contribution from the prefrontal cortex. This is somewhat in line with the present results as minimal involvement was observed from the different regions of the PFC, as evidenced by the low

concentration of HbO and increased concentration of HbR. Only the orbitofrontal region of the PFC exhibited higher concentration of HbO when compared to the mPFC.

There are a few limitations of note with the present study. The first was the short practice period. It is possible that greater learning benefits could have been observed if an environment with increased contextual interference was provided (Shea & Morgan, 1979). This would have optimized learning by forcing participants to use multiple processing strategies.

Second, the observed differences in the data may have been influenced by the characteristics of the fNIRS signal. Compared to fMRI, the signal-to-noise ratio (SNR) of fNIRS is low (Cui, Bray, Bryant, Glover, & Reiss, 2011). This could have affected its ability to detect similar patterns as Wu et al. (2004). However, we did take steps to improve the SNR and minimize this limitation by maintaining a block design (Cui et al., 2011; Scarapicchia et al., 2017) and applying a PCA (Cooper et al., 2009). In addition, physiological signals such as cardiac rhythm, respiration, and Mayer waves arising from the scalp and underlying cerebral tissue are known to be significant contributors of signal noise (Huppert et al., 2009; Yucel et al., 2016). Attempts to eliminate background physiological signals were also made by applying a bandpass filter between 0.01 and 0.5 Hz. However, due to their low frequency characteristics and similarity to the hemodynamic response, contributions from extra-cerebral tissue, skin blood flow, Mayer waves, and respiration were still present in the analyzed signal (Hoshi, 2016; Yucel et al., 2016). This may explain why the variability across conditions was sometimes twice the mean. Third, the choice of filter could have influenced the accuracy of the evoked hemodynamic response (Pfeifer, Scholkmann, & Labruyere, 2018). For this study, steps were taken to ensure an appropriate correction method was employed. The three most commonly used corrections (wavelet analysis, spline interpolation and PCA) (Cooper et al., 2012; Huppert et al., 2009) were

applied to the signal during the pre-processing stage. Out of all the corrections, PCA reduced a considerable amount of variability and recovered the most representative hemodynamic response. Fourth, the fixed distance between the source and detector diodes could have contributed to the differences in findings, since the penetration depth of infrared light is dependent on it (Ferrari & Quaresima, 2012; Quaresima & Ferrari, 2019; Scarapicchia et al., 2017). As well as the variability in scalp to brain distance across participants and the technology's poor spatial localization (Cui et al., 2011; Quaresima & Ferrari, 2019). Lastly, while in some comparisons we did not find expected differences in HbR, this could be attributed to the fact that HbR is not as sensitive as HbO to task-related changes (Miyai et al., 2001). Specifically, when using optical means, HbO is a more sensitive measure to changes in cerebral blood flow, whereas the concentrations of HbR can be influenced by venous blood oxygenation and volume (Hoshi, Kobayashi, & Tamura, 2001).

2.6. Conclusion

In conclusion, the present findings were unable to replicate the reduction in cortical activation in the left DLPFC, following learning as reported by Wu et al. (2004). Although behavioural improvements were observed, it is argued that the practice period did not allow for adequate time to learn the motor sequences to the point that neural connectivity in the PFC was greatly strengthened. However, the increased contribution from the right hemisphere did reveal the potential involvement of working memory in motor sequence production and the lateralization associated with explicit learning.

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3. Chapter 3

Involvement of the prefrontal cortex in motor sequence learning: A functional near-infrared spectroscopy (fNIRS) study²

² A version of this chapter is currently in preparation for resubmission:

Polskaia, N., St-Amant, G., Fraser, S., & Lajoie, Y. (In preparation). Involvement of the prefrontal cortex in motor sequence learning: A functional near-infrared spectroscopy (fNIRS) study.

3.1. Abstract

Our previous functional near-infrared spectroscopy (fNIRS) study on motor sequence learning (Polskaia et al., 2020) was unable to replicate the decrease in left dorsolateral prefrontal cortex activity (DLPFC) associated with movement automaticity, as reported by Wu et al. (2004). We suggest that learning of the motor sequence skill was not achieved due to the short practice period (2-4 hours). We sought to expand on our previous work to better understand the contribution of the prefrontal cortex (PFC) to motor sequence learning by including additional practice sessions, a retention phase, and a control group. Participants were randomly assigned to one of two experimental groups: control or trained. fNIRS was acquired at three time points: pre-test (day 1), post-test (day 4), and retention (day 5). Participants were required to perform four sequences (S1, S2, S3, and S4) of right hand finger-tapping. In addition, the trained group underwent four days of practice of S1 and S2. For S1 and S2, no group differences in activation were observed in the left DLPFC and ventrolateral (VLPFC) between sessions. The control group exhibited increased error scores in retention compared to the trained group. However, no significance was found between pre-test and retention from the trained group. Increased contribution of the right VLPFC was observed in post-test for the trained group, which may reflect the active retrieval of explicit information from long-term memory. For S3 and S4, no group differences in cortical activation were observed. Our results suggest that explicit motor sequence learning requires the continued involvement of the PFC.

3.2. Introduction

Functional imaging studies have shown structures such as the prefrontal cortex (PFC), the primary motor cortex (M1), premotor cortex (PMC), supplementary motor area (SMA), cerebellum, and basal ganglia to be involved in motor sequence learning (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Doyon et al., 2002; Eliassen, Souza, & Sanes, 2001; Grafton, Hazeltine, & Ivry, 1995; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Karni et al., 1995; Lehericy et al., 2005; Penhune & Doyon, 2002; Steele & Penhune, 2010; Toni, Krams, Turner, & Passingham, 1998; Wu, Kansaku, & Hallett, 2004). It is suggested that depending on the phase of acquisition (early vs. late), motor skill learning is supported by two distinct neural networks: cortico-cerebellar and cortico-striatal (see Lohse, Wadden, Boyd, & Hodges, 2014 for review). The early stage of learning is predominately represented by increased contributions of the cerebellum, frontal lobe, parietal cortex, and premotor cortex (Doyon et al., 1996; Jenkins et al., 1994; Penhune & Doyon, 2002; Steele & Penhune, 2010). The late stage of learning is predominately represented by reduced activation of the cerebellum and increased contributions of the M1, SMA, and basal ganglia (Doyon et al., 1996; Grafton et al., 1995; Jenkins et al., 1994; Karni et al., 1995; Lehericy et al., 2005; Penhune & Doyon, 2002; Steele & Penhune, 2010). The PFC has been found to be involved in both stages of the learning process. Neuroimaging studies have demonstrated PFC activation to decrease with practice suggesting that its involvement is minimally required when the task is performed automatically (Jenkins et al., 1994; Toni et al., 1998; Wu et al., 2004). Conversely, studies have also observed increased activity in the late stage of learning suggesting greater involvement of the PFC is required when sequential tasks are explicitly learned and have working memory requirements (Doyon et al., 1996; Doyon et al., 2002; Eliassen et al., 2001; Grafton et al., 1995; Hazeltine, Grafton, & Ivry, 1997; Penhune &

Doyon, 2002; Steele & Penhune, 2010). Interestingly, only the latter was observed during our evaluation of movement automaticity (Polskaia et al., 2020). Findings revealed sequence production to have a significant increase in the right dorsolateral PFC (DLPFC) with no significant decrease in activation in the left hemisphere, as would be expected with automaticity. The latter finding was attributed to a short practice period that did not provide adequate time for motor learning to be established.

Of the neuroimaging studies, very few have included a retention phase in their sequence learning protocols (Penhune & Doyon, 2002; Steele & Penhune, 2010). A retention test is necessary to distinguish learning from performance by evaluating the persistence of a motor skill (Schmidt & Lee, 2011). The lack of retention tests in neuroimaging studies may in part be related to the high cost associated with fMRI or PET (Lohse et al., 2014). Functional near-infrared spectroscopy (fNIRS) is a neuroimaging technique that can enable long-term evaluation due to its low cost and portability (Bunce, Izzetoglu, Izzetoglu, Onaral, & Pourrezaei, 2006; Quaresima & Ferrari, 2019). fNIRS provides an indirect and non-invasive method for examining oxygenation changes in the brain (Pinti, Scholkmann, Hamilton, Burgess, & Tachtsidis, 2019; Quaresima & Ferrari, 2019). It can quantify the relative concentrations of oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and total hemoglobin (tHb) by capturing changes in light intensity in the near-infrared range (700 - 900 nanometers (nm)) (Bunce et al., 2006; Scholkmann et al., 2014). Although its spatial resolution is limited to a 2 – 3 centimeter range, fNIRS provides a higher temporal resolution than fMRI (Perrey, 2008). Additionally, fNIRS has a higher tolerance to motion artifacts, better resolution of the hemodynamic onset and enables neuroimaging in more ecologically valid environments (Perrey, 2008; Pinti et al., 2018). For instance, cerebral oxygenation has been monitored in various cortical regions (e.g., M1, PFC,

SMA, PMC, and primary sensorimotor cortex) during motor and cognitive tasks such as finger-tapping, finger to thumb opposition, walking, running, word generation, n-back, and Stroop (see Leff et al., 2010; Obrig & Villringer, 2003 for review). However, to our knowledge, few fNIRS studies have examined the role of the PFC in motor sequence learning (Immink, Pointon, Wright & Marino, 2021; Lu & Yuan, 2015; Polskaia et al., 2020).

Therefore, using fNIRS to quantify changes in oxygenation, the objective of the present study was to build on our previous work (Polskaia et al., 2020) by including more practice sessions, a retention period, and a control group (Penhune & Doyon, 2002; Steele & Penhune, 2010) in order to better understand the contribution of the PFC to motor sequence learning and movement automaticity. To do so, we monitored a training group over five consecutive days (four days of practice followed by a retention test) and compared their performance to a control group that underwent no motor sequence learning. It was predicted that if learning was achieved, the trained group would exhibit decreased activity in the left hemisphere between pre-test and retention, with no changes in activation observed in the control group. Additionally, it was predicted that explicit sequence learning and active retrieval of explicit knowledge would be represented by increased right hemisphere activation between pre-test and retention for the trained group only.

3.3. Materials and methods

3.3.1. Participants

Fourteen, right-handed, young adults were recruited to participate in the study (24.71 ± 2.05 years). Participants provided written informed consent, which was approved by the

University of Ottawa Ethics Committee. Participants were excluded if they had any self-reported neurological and/or visual impairments.

3.3.2. Functional near-infrared spectroscopy (fNIRS)

PFC activation was evaluated through task-related changes in concentration of HbO and HbR using a multichannel, continuous-wave spectrometer (Octamon, Artinis Medical Systems, Einsteinweg, Netherlands). The data was collected at a sampling rate of 10 Hz. The device was equipped with eight light emitting diodes (760 and 850 nm) and two photodiode detectors, resulting in an 8-channel system (channels T1-T8). The optodes were secured onto a headband with channels T1-T4 situated over the right hemisphere and channels T5-T8 over the left hemisphere. The optode distance was fixed at 35 mm. The modified 10/20 positioning system was used to ensure proper optode placement over Fp1 and Fp2 (Niedermeyer & Lopes de Silva, 2004). Although the optodes were not coregistered to an MNI brain template, it was presumed that based on their placement, T1/T7 were located over the dorsolateral PFC (DLPFC), T2/T8 over the ventrolateral PFC (VLPFC), T3/T5 over the medial PFC (mPFC), and T4/T6 over the orbitofrontal cortex (Figure 3.1 AB). Due to its greater sensitivity to task-related changes, only relative changes in HbO concentration will be reported (Miyai et al., 2001).

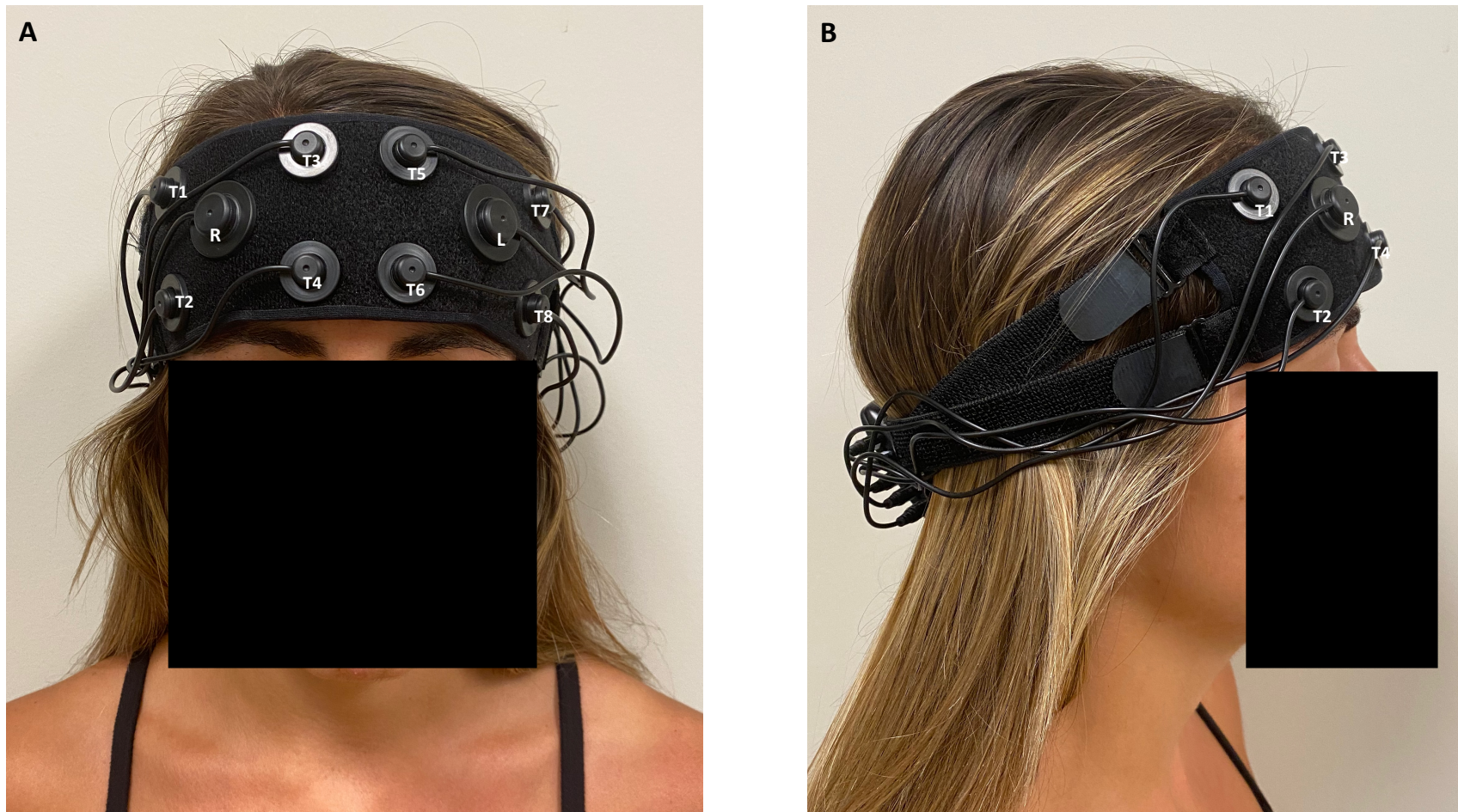


Figure 3.1. fNIRS configuration. **(A)** Anterior view of the right and left hemisphere diodes. T1 – T4: right hemisphere light emitting diodes. R: right hemisphere detector. T5 – T8: left hemisphere light emitting diodes. L: left hemisphere detector. **(B)** Sagittal view of the right hemisphere diodes.

3.3.3. Additional apparatus

A child's miniature piano was repurposed to serve as the key press device. This response device was used to record participants' sequence production (e.g., number of key presses and frequency of responding). All key presses were transmitted to a computer equipped with National Instrument data acquisition software. Visual signals for active and rest blocks were presented using a 15" laptop.

3.3.4. Sequences

Participants were instructed to perform four sequences of right handed finger-tapping. Each sequence was eight units in length. Sequence-1 (S1) consisted of 5-1-2-5-4-2-4-3, in which, 1, 2, 3, 4, and 5 corresponded to the thumb, index, middle, ring, and pinky fingers, respectively. Sequence-2 (S2) consisted of 4-2-1-3-5-3-1-4, sequence-3 (S3) consisted of 1-4-2-5-3-2-5-1, and sequence-4 (S4) consisted of 2-2-3-1-4-3-5-2. To capture changes in tapping performance, participants' speed was not regulated during any stage of performance (i.e., familiarization, acquisition, and practice). Therefore, the amount of times the sequences were completed during an active condition varied across participants. Participants were randomly assigned to one of two experimental groups: control or trained. For the control group, all four sequences were performed during the pre-test, post-test, and retention. The trained group completed the same sequences as the control group and was required to learn two of the four sequences (S1 and S2) across four days of practice. The entire protocol was carried out across five consecutive days (Figure 3.2A). The practice sessions were completed in a seated position.

3.3.5. fNIRS acquisition

fNIRS data was acquired during pre-test (day 1), post-test (day 4), and retention (day 5). During the pre-test and post-test sessions, participants from both groups were given a brief familiarization period (approximately 5-10 minutes) before the acquisition of each sequence. The acquisition sessions consisted of the four sequences (S1-S4) in an ON-OFF cycle paradigm of rest (baseline) and active (experimental task) (Figure 3.2B). For each sequence, rest and active was 20-seconds in duration and repeated four and three times, respectively. For the rest blocks, participants were instructed to relax and focus on the fixation cross displayed on the screen. This enabled any extraneous stimuli to be accounted for. For the active blocks, participants were instructed to perform S1, S2, S3 or S4. Feedback on performance was not provided during the testing periods. Acquisition was completed in a seated position.

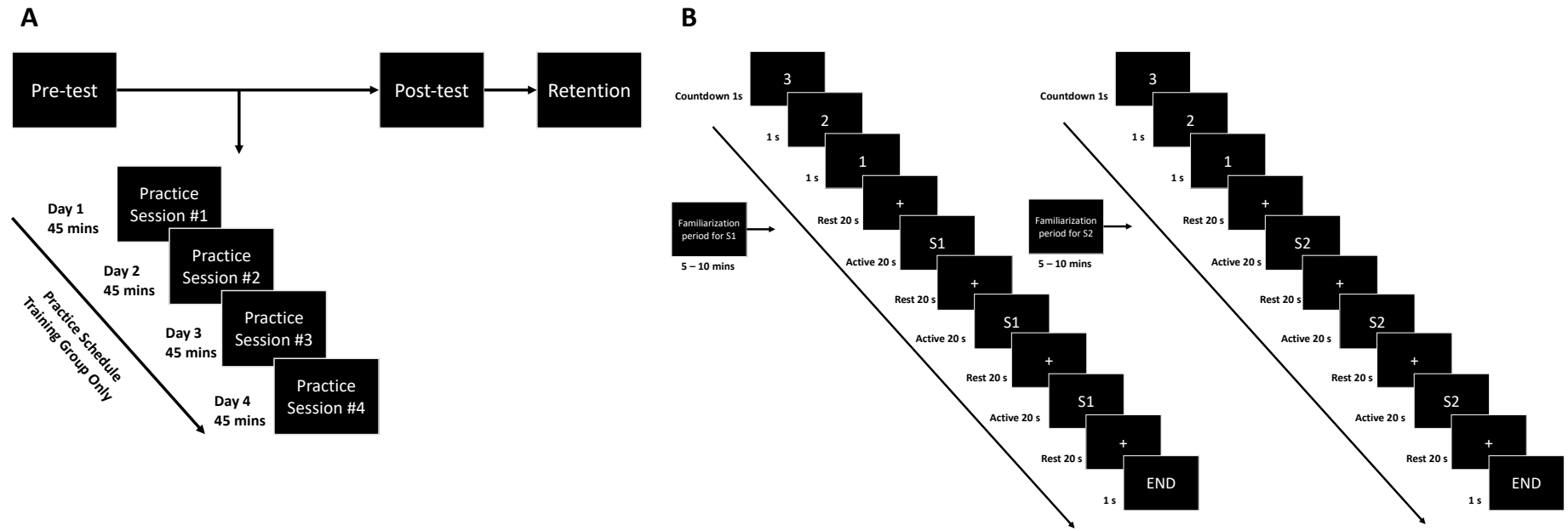


Figure 3.2. Schematic of the experimental paradigm. **(A)** Illustration of the experimental protocol for the trained and control groups. Both groups were evaluated at three time points: pre-test, post-test, and retention. The trained group underwent four days of practice. The entire experimental protocol took five days to complete. **(B)** Illustration of the fNIRS acquisition for S1 and S2. The same structure was used for S3 and S4. Acquisition was always preceded by a 5-10 minute familiarization period. Each sequence was performed in an ON-OFF cycle paradigm of rest and active. Each acquisition began with a countdown and finished with an “END” title slide. The sequences were presented in the following order: S1, S2, S3, and S4.

3.3.6. Practice

Only participants assigned to the trained group were required to attend the practice sessions. The practice sessions were performed across four consecutive days and were 45 minutes in duration. On day one, practice occurred 15 minutes after the pre-test. On day four, the post-test was completed 15 minutes after the final practice session. Participants were required to only learn S1 and S2. The sequences were practiced in 20-second blocks using a randomized practice schedule. After 15 blocks, participants received summary knowledge of results (KR) consisting of the number of blocks with errors. For each practice session, summary KR was provided a total of nine times.

3.3.7. Data processing

Data processing and analysis was performed with HomER (version 2), a MATLAB-based graphical user interface program designed for processing functional cortical data acquired from NIRS (Huppert, Diamond, Franceschini, & Boas, 2009). The processing stream and parameters were derived from Brigadoi et al. (2014), Cooper et al. (2012), and Di Lorenzo et al. (2019). First, the raw intensity data was converted to optical density (`hmrIntensity2OD`). Then a wavelet analysis (`hmrMotionCorrectWavelet`) with an interquartile range of 0.5 (Di Lorenzo et al. 2019) was applied to detect and reduce the presence of motion artifacts. Any remaining uncorrected motion artifacts were then identified and removed using the `hmrMotionArtifact` function. Specifically, if the signal exceeded an optical density of 0.4 (`AMPthresh`) or a standard deviation of 20 (`SDthresh`) over 1-second (`tMotion`), it was masked for 1-second before and after (`tMask`) (Di Lorenzo et al., 2019; Brigadoi et al., 2014; Cooper et al. 2012). Next, a fourth-order

Butterworth bandpass filter was applied to remove any additional sources of noise (e.g., physiological noise and instrumentation) (hmrBandpassFilt). A low-pass filter of 0.5 Hz was applied to remove high-frequency instrument noise and cardiac oscillations (Huppert et al., 2009). A high-pass filter of 0.01 Hz was applied to remove any low-frequency drift (Cooper et al., 2012). The data was then converted (hmrOD2Conc) from optical density to relative concentration using the modified Beer-Lambert law (Villringer & Chance, 1997). Lastly, a block average (hmrBlockavg) over a time period of -10-seconds and 20-seconds was calculated for each sequence. The baseline of the average was set to zero by subtracting the mean of $t < 0$ from $t > 0$.

3.3.8. Dependent variables and statistical analysis

Sequence production was recorded to track errors and frequency of responding. A wrong key press was characterized as an error. The number of key presses generated during a 20-second active block was used to calculate frequency of responding (Hz). Cortical activation was represented by the relative change in concentration of HbO across the active condition. The HRF was block averaged to increase the signal-to-noise ratio and reliability of the fNIRS signal (Gervain et al., 2011).

In terms of statistical analysis, a group (control, trained) x session (pre-test, post-test, retention) x sequence (S1, S2) x channel (T1, T2, T3, T4, T5, T6, T7, T8) mixed analysis of variance (ANOVA) was performed on HbO with repeated measure (RM) on the last three factors. Similarly, a group x session x sequence (S3, S4) x channel mixed ANOVA was performed on HbO. For the behavioural data, two separate mixed RM ANOVAs were performed for error score and frequency of responding. A group x time x sequence (S1, S2) and a group x

time x sequence (S3, S4) with RM on the last two factors were performed. When required, Bonferroni was used to determine the location of significance. Statistical significance was set to $p < 0.05$.

3.4. Results

3.4.1. Behavioural data

All behavioural data is reported in Table 3.1.

Table 3.1.

Error score and frequency of responding (Hz) data for the control and trained groups across three testing sessions

Sequence	Errors						Frequency of Responding (Hz)					
	Control Group			Trained Group			Control Group			Trained Group		
	Pre-test	Post-test	Retention	Pre-test	Post-test	Retention	Pre-test	Post-test	Retention	Pre-test	Post-test	Retention
S1	1.19 ±	0.24 ±	16.57 ±	1.81 ±	0.096 ±	0.090 ±	1.96 ±	2.45 ±	1.86 ±	2.34 ±	4.85 ±	4.37 ±
	2.43	0.25	12.51	2.28	0.25	0.15	0.88	1.17	0.89	0.74	1.93	1.43
S2	0.24 ±	0.48 ±	18.86 ±	0.67 ±			1.83 ±	2.12 ±	1.95 ±	1.89 ±	4.39 ±	4.71 ±
	0.25	0.98	15.87	0.58	0	0	1.01	1.02	1.00	0.61	1.48	1.96
S3	0.19 ±	0.47 ±	17.95 ±	0.47 ±	0.52 ±	29.71 ±	1.80 ±	2.27 ±	1.70 ±	2.00 ±	2.77 ±	2.00 ±
	0.26	0.83	12.69	0.83	0.94	24.99	0.86	1.10	0.84	0.63	0.85	0.98
S4	0.14 ±	0.29 ±	19.05 ±	0.48 ±	0.68 ±	7.38 ±	2.01 ±	2.18 ±	1.76 ±	2.05 ±	2.99 ±	3.12 ±
	0.26	0.41	17.70	0.57	0.96	11.00	0.79	0.92	0.71	0.77	0.83	1.30

Note. Pre-test, post-test, and retention were performed on day 1, day 4, and day 5, respectively. Values are mean ± SD. Errors were classified as incorrect finger presses. Frequency of responding = total number of key presses generated during an active block/20-seconds.

3.4.1.1. Error score (S1 and S2)

The main effect of group, $F(1, 12) = 10.74, p < 0.01, \eta^2 = 0.472$, and session, $F(2, 24) = 10.93, p < 0.01, \eta^2 = 0.477$, were superseded by a group x session interaction, $F(2, 24) = 12.50, p < 0.001, \eta^2 = 0.510$. The post-hoc analysis revealed an increase in errors between pre-test and retention for the control group ($p < 0.05$). The main effect of sequence and all remaining interactions failed to reach statistical significance ($p_s > 0.05$).

3.4.1.2. Error score (S3 and S4)

The ANOVA revealed a significant main effect of session, $F(2, 24) = 28.72, p < 0.001, \eta^2 = 0.705$. According to the post-hoc analysis, participants committed significantly more errors during retention when compared to pre-test ($p < 0.001$) and post-test ($p < 0.01$). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

3.4.1.3. Frequency of responding (S1 and S2)

The main effect of group, $F(1, 12) = 9.13, p < 0.05, \eta^2 = 0.432$, and session, $F(2, 24) = 19.71, p < 0.001, \eta^2 = 0.622$, were superseded by a group x session interaction, $F(2, 24) = 14.12, p < 0.001, \eta^2 = 0.541$. The post-hoc analysis revealed that the trained group exhibiting significantly higher frequency of responding during retention ($p < 0.05$) compared to pre-test ($p < 0.05$) and post-test ($p < 0.05$). The ANOVA revealed a significant session x sequence interaction, $F(2, 24) = 4.79, p < 0.05, \eta^2 = 0.285$. The post-hoc analysis revealed: 1) a higher frequency of responding during post-test ($M = 3.65, SD = 1.97$) compared to pre-test ($M = 2.15, SD = 0.81$) (p

< 0.01) and retention ($M = 3.11$, $SD = 1.74$) ($p < 0.05$) for S1; and 2) a lower frequency of responding during pre-test ($M = 1.86$, $SD = 0.80$) compared to post-test ($M = 3.26$, $SD = 1.70$) ($p < 0.001$) and retention ($M = 3.33$, $SD = 2.07$) ($p < 0.01$) for S2. All remaining interactions failed to reach statistical significance ($p_s > 0.05$).

3.4.1.4. Frequency of responding (S3 and S4)

The main effect of session, $F(2, 24) = 14.38$, $p < 0.001$, $\eta^2 = 0.545$, sequence, $F(1, 12) = 10.20$, $p < 0.01$, $\eta^2 = 0.460$, and session x sequence, $F(2, 24) = 6.44$, $p < 0.01$, $\eta^2 = 0.349$, were superseded by a group x session x sequence interaction, $F(2, 24) = 7.33$, $p < 0.01$, $\eta^2 = 0.379$. The post-hoc analysis revealed for the trained group: 1) higher frequency of responding during post-test compared to pre-test ($p < 0.05$) and retention ($p < 0.05$) for S3; and 2) lower frequency of responding during pre-test ($p < 0.05$) compared to post-test ($p < 0.05$) and retention ($p < 0.05$) for S4.

3.4.2. Cortical activation (limited to HbO only)

Negative concentrations of HbO were observed in the present data. Preparation for an upcoming motor response may have triggered greater activation during rest than active (Issard & Gervain, 2018).

3.4.2.1. S1 and S2

The main effect of channel, $F(7, 84) = 5.82$, $p < 0.01$, $\eta^2 = 0.327$, sequence x channel, $F(7, 84) = 2.50$, $p < 0.05$, $\eta^2 = 0.172$, and group x session x channel, $F(14, 168) = 1.98$, $p < 0.05$, $\eta^2 = 0.142$, were superseded by a group x session x channel x sequence interaction, $F(14, 168)$

$= 1.85, p < 0.05, \eta p^2 = 0.134$. The post-hoc analysis revealed: 1) an increase in cortical activity in the right VLPFC (T2) during post-test ($M = 0.175 \mu M, SD = 0.211$) compared to pre-test ($M = -0.058 \mu M, SD = 0.205$) for the trained group for S1 ($p < 0.01$); 2) a decrease in cortical activity in the right VLPFC (T2) during retention ($M = -0.064, SD = 0.149$) compared to pre-test ($M = 0.131 \mu M, SD = 0.160$) and post-test ($M = -0.025 \mu M, SD = 0.199$) for the control group for S1 ($p_s < 0.05$); 3) a decrease in cortical activity in the right orbitofrontal cortex(T4) during retention ($M = -0.051 \mu M, SD = 0.152$) compared to pre-test ($M = 0.227 \mu M, SD = 0.111$) for the control group for S1 ($p < 0.05$); and 4) a decrease in cortical activity in the right orbitofrontal cortex during post-test ($M = -0.122 \mu M, SD = 0.139$) compared to pre-test ($M = 0.167 \mu M, SD = 0.147$) for control group for S2 ($p < 0.05$). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

3.4.2.2. S3 and S4

The main effect of channel, $F(7, 84) = 5.03, p < 0.001, \eta p^2 = 0.296$, was superseded by a session x channel x sequence interaction, $F(14, 168) = 2.20, p < 0.05, \eta p^2 = 0.155$. The post-hoc analysis revealed a decrease in cortical activity in the left medial PFC (T5) during retention ($M = -0.072 \mu M, SD = 0.111$) compared to post-test ($M = 0.067 \mu M, SD = 0.128$) for S3 ($p < 0.05$). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

3.5. Discussion

The aim of this study was to expand on our previous work (Polskaia et al., 2020) to identify the neural correlates of motor sequence learning and performance in the PFC using fNIRS. Our hypothesis was not supported, as no group differences were observed in the left

DLPFC and VLPFC between sessions. Findings revealed increased activation of the right VLPFC in post-test for the trained group, which may reflect the tasks' working memory and explicit knowledge requirements. With limited behavioural and cortical findings, the present study was unable to differentiate learning from performance.

Behavioural data

Error scores for S1 and S2 were not significantly different across groups for pre-test and post-test (Table 3.1). We attribute this similarity, especially in the post-test session, to the familiarization period completed prior to acquisition. We suspect participants, particularly in the control group, were able to rehearse and briefly retain the sequences in short-term memory and as a result, perform them with minimal errors. In retention, however, a notable difference in error score was observed between groups (Table 3.1). As expected, the control group produced significantly more errors, which suggests that sequential knowledge was not retained. The trained group, on the other hand, demonstrated similar performance between pre-test and retention. As a result, the present findings cannot definitively indicate if practice influenced performance in a relatively permanent way (Schmidt & Lee, 2011) and if movement automaticity was achieved.

For S3 and S4, both groups committed significantly more errors in retention compared to pre-test. This was to be expected, as the design of the study did not enable learning of either sequence.

Additionally, participants' frequency of responding was recorded to determine if learning-related changes could be observed. For S1 and S2, the trained group exhibited higher frequency during post-test and retention compared to the control group. This suggests that

practice can enable faster movement production, while simultaneously maintaining accuracy. This is consistent with several motor sequence studies that have reported increased speed after days or weeks of practice (Karni et al., 1995; Lehericy et al., 2005). However, due to the error score data, it is unclear if these changes in performance are indicative of relative permanence or a temporary practice effect. For S3 and S4, higher frequency of responding was observed during post-test and retention, respectively, for the trained group. This may be a consequence of the familiarization period, or a result of strategies developed during practice. Conversely, the control group maintained a relatively similar frequency across sessions for S3 and S4.

Activation patterns associated with learning

We found no significant difference in activation in the left DLPFC between sessions for both groups (refer to Supplementary Table 3.2 and 3.3). This is contrary to several neuroimaging studies showing increased DLPFC activation in the early stage of learning, but not in the late/automatic stage when movement automaticity is achieved (Jenkins et al., 1994; Jueptner et al., 1997; Toni et al., 1998; Wu et al., 2004). Considering the similarity in activation between groups in retention, we suggest that activation of this region may not reflect movement automaticity but rather attentional investment in movement preparation of previously learned sequences (Jueptner et al., 1997; Sakai, Ramnani, & Passingham, 2002). Jueptner et al. (1997) reported reactivation of the left DLPFC and right anterior cingulate cortex only when participants were instructed to direct attention to the performance of a pre-learned sequence. Conversely, when participants were instructed to perform the pre-learned sequence with no attentional direction, activation of the DLPFC and anterior cingulate cortex was not observed.

This will need to be confirmed in future studies to determine if it contradicts previous work on movement automaticity (Jenkins et al., 1994; Toni et al., 1998; Wu et al., 2004).

Comparably, no differences in activation were detected in the left VLPFC between sessions for the control and trained groups (refer to Supplementary Table 3.2 and 3.3). The VLPFC has been implicated in the control (e.g., selection and maintenance) of stimulus information needed to guide motor behaviour (Karim et al., 2018). Given that stimulus information was only required to initiate motor performance, this could explain why minimal activation was observed in both groups.

For the trained group, activity in the right VLPFC was significantly higher in the post-test compared to the pre-test for S1. Conversely, the opposite was observed for the control group, with reduced activity in the right VLPFC in the post-test and retention (Figure 3.3). The observed increase in VLPFC activity is consistent with several neuroimaging studies on motor sequence learning (Doyon et al., 1996; Doyon et al., 2002; Penhune and Doyon, 2002). The VLPFC, in connection with posterior cortical association areas, is suggested to be involved in working memory and the active retrieval of explicit information from long-term memory (Doyon et al., 1996; Eliassen et al., 2001; Petrides, 1994; Petrides, 1996; Petrides, 2002). This could explain why opposing patterns of activation were observed between the groups in post-test. However, with significance limited to the post-test session, the increased activation may reflect relative permanence or temporary effects associated with practice (Kantak & Winstein, 2012). This observation is consistent with the behavioural data discussed above.

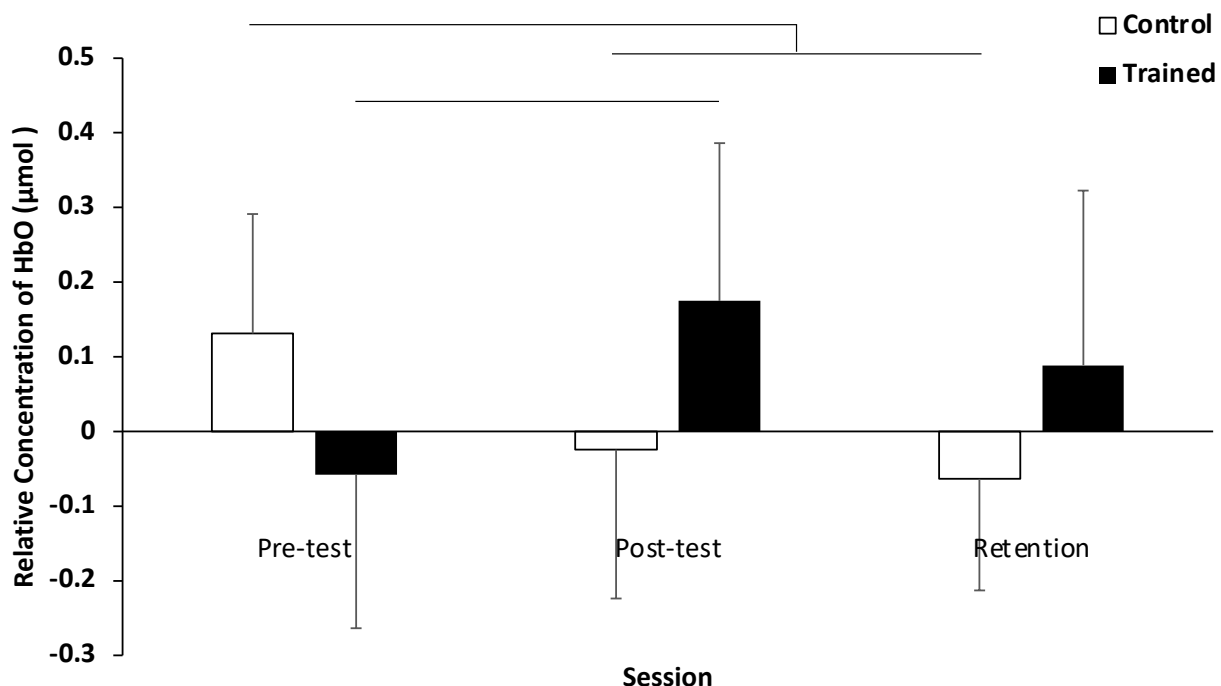


Figure 3.3. Change in relative concentration of HbO (mean $\pm$ SD) in the right ventrolateral prefrontal cortex (VLPFC) for the control and trained groups as a function of session. Horizontal lines represent statistical significance ($p < 0.05$).

The findings are also consistent with the suggestion that the ipsilateral PFC is involved in explicit motor sequence learning (Doyon et al., 1996; Grafton et al., 1995; Hazeltine et al., 1997). The aforementioned studies have reported cortical activation to differ depending on the type of knowledge used during motor learning. Implicit knowledge has been shown to involve cortical regions such as the striatum, putamen, cerebellum, and supplementary motor area, while explicit knowledge has required the involvement of the prefrontal cortex, parietal cortex, and premotor cortex.

For the control group, the present findings revealed reduced activity in the right orbitofrontal cortex during post-test and retention for S2 and S1, respectively (Figure 3.4). The

opposite functional changes were observed in the trained group, however, the findings failed to reach significance. This is consistent with several neuroimaging studies showing increased involvement of the orbitofrontal cortex during acquisition of sequential movements (Jackson, Lafleur, Malouin, Richards, & Doyon, 2003; Lafleur et al., 2002; Penhune & Doyon, 2002; Polskaia et al., 2020). Additionally, the medial orbitofrontal cortex has been suggested to be involved in selecting stimuli and/or responses based on their correctness or reward value (Elliott, Dolan & Frith, 2000). Therefore, the reduced activity in the right orbitofrontal cortex may reflect the absence of motor sequence learning and the generation of responses with no intrinsic value.

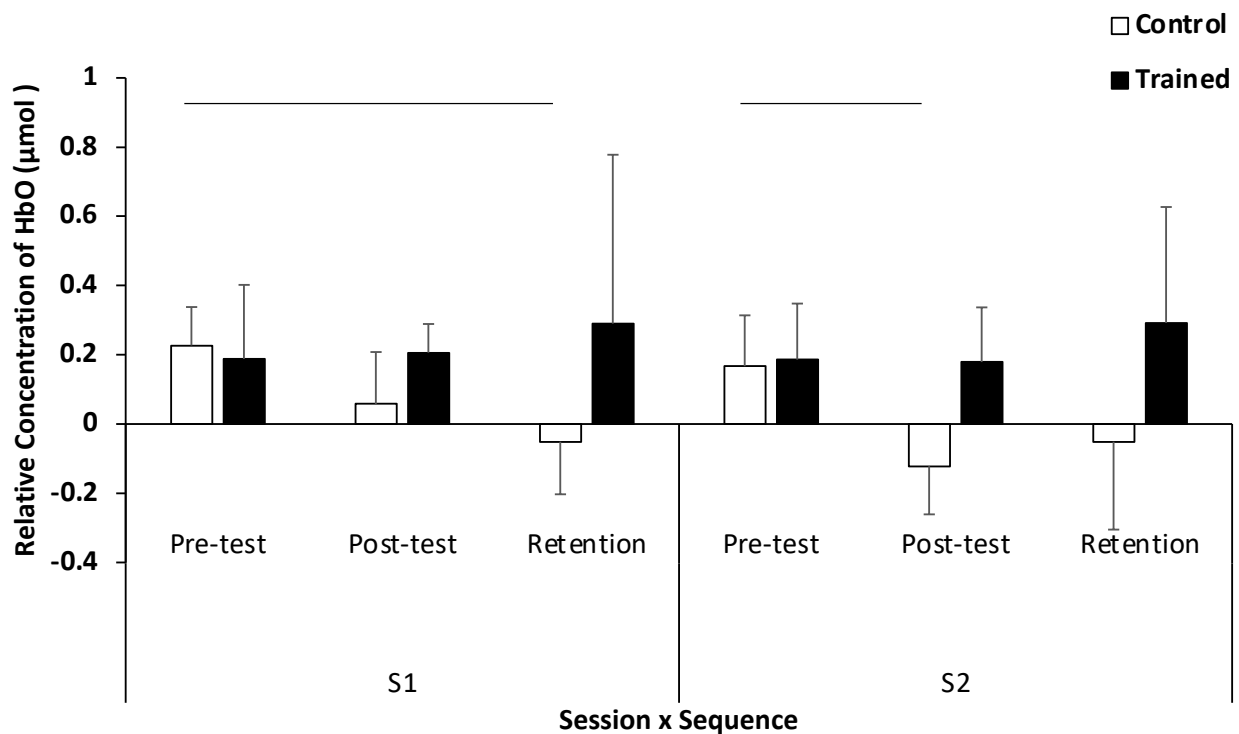


Figure 3.4. Change in relative concentration of HbO (mean $\pm$ SD) in the right orbitofrontal cortex for the control and trained groups as a function of session and sequence. Horizontal lines represent statistical significance ($p < 0.05$).

Activation in the absence of learning

The present findings revealed no significant difference between the control and trained groups for S3 and S4. This is consistent with the error score data (Table 3.1). Significance was limited to S3, with reduced activity in the left mPFC in retention compared to post-test (Figure 3.5). Neuroimaging studies have linked the mPFC to explicit sequence learning (Destrebecqz et al., 2003), error processing (Menon, Adelman, White, Glover & Reiss, 2001), and long-term memory (Miller & Cohen, 2001). Reduced activity in the mPFC may suggest that participants did not engage in any of the mechanisms that would typically be required for sequential learning. The high error scores in retention may further support the suggestion that participants did not undergo learning. Additionally, reduced activation was observed in the right VLPFC, bilateral orbitofrontal cortex, left DLPFC and left VLPFC in retention (Refer to Supplementary Table 3.4 and 3.5). Although significance was not reached in these regions, it may still provide some support to the motor learning-related observations made in the previous section of the discussion.

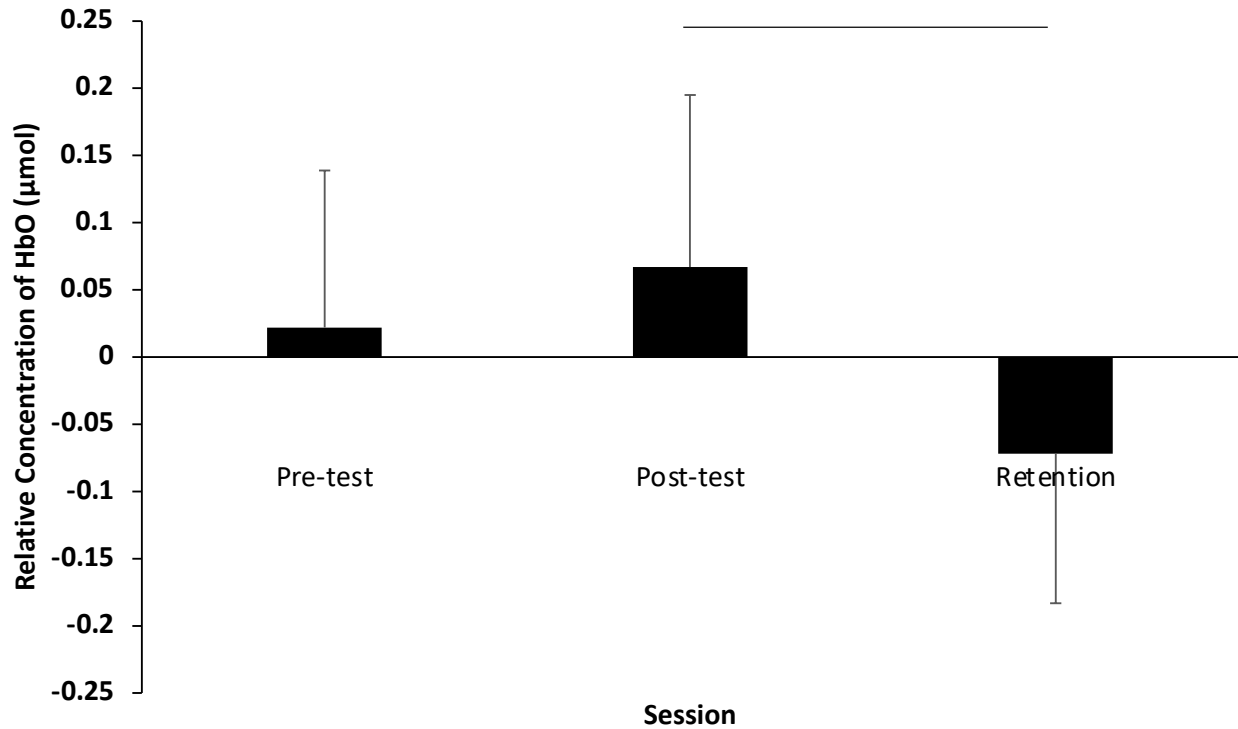


Figure 3.5. Change in relative concentration of HbO (mean $\pm$ SD) in the left medial prefrontal cortex (mPFC) as a function of session. Horizontal line represents statistical significance ($p < 0.05$).

Limitations

There are a few limitations with the present study. First, the influence of systemic activity on the variability of the fNIRS signal (Tachtsidis & Scholkmann, 2016). Responses such as heart rate, respiration, arterial pulse, and autonomic nervous system activity have been shown to interfere with the fNIRS signal or even compromise the evoked HRF (Tachtsidis & Scholkmann, 2016; Zhang et al., 2005). They can exhibit fluctuations that are also temporally correlated with the neuronal response. Finger-tapping, in particular, has been found to precipitate a synchronization between the task's stimulation frequency and systemic activity such as heart rate, respiration, and arterial pulse (Franceschini, Fantini, Toronov, Filiaci, & Gratton, 2000). In

addition to task modality, spontaneous low-frequency oscillations in response to neuronal activity may have also contributed to our observed variability (Holper et al., 2012; Hu, Hong, & Ge, 2013). Second, the fNIRS signal may have contained motion artifacts comparable in frequency and amplitude to the hemodynamic response function (Brigadoi et al., 2014). The wavelet function relies on the assumption that motion artifacts are different from the hemodynamic signal in frequency and duration (Molavi & Dumont, 2012). If motion artifacts were similar in profile to the HRF, the wavelet algorithm could have potentially attenuated the HRF by assigning coefficients of zero to those data points (Brigadoi et al., 2014; Di Lorenzo et al., 2019). Finally, the low signal-to-noise ratio of fNIRS (Cui, Bray, Bryant, Glover, & Reiss, 2011) may have compromised its ability to detect differences in activation.

3.6. Conclusion

The behavioural findings were unable to demonstrate if movement automaticity was achieved. Similar observations were made with regards to the activation observed in left DLPFC and VLPFC. The significant increase in right VLPFC activity in the trained group may reflect explicit motor sequence learning and memory retrieval. Furthermore, the role of the orbitofrontal cortex in sequential movements and reward, and the mPFC in explicit learning and error processing, may explain why reduced activations were observed in the control group and in the absence of motor sequence learning (S3 and S4), respectively.

3.7. References

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3.8. Supplementary material

Table 3.2.

Mean relative concentration change of HbO ($\pm$ SD) for each region of interest during S1 production across groups and sessions

Regions of interest	Pre-test		Post-test		Retention	
	Control group	Trained group	Control group	Trained group	Control group	Trained group
<i>Right hemisphere</i>						
Dorsolateral PFC	0.041 (0.17)	0.036 (0.11)	0.111 (0.16)	0.070 (0.25)	0.021 (0.15)	0.084 (0.25)
Ventrolateral PFC	0.131 (0.16)	-0.058 (0.21)	-0.024 (0.20)	0.175 (0.21)	-0.064 (0.15)	0.089 (0.23)
Medial PFC	0.147 (0.26)	0.092 (0.16)	0.100 (0.16)	0.173 (0.11)	0.059 (0.17)	0.067 (0.26)
Orbitofrontal Cortex	0.227 (0.11)	0.188 (0.21)	0.058 (0.15)	0.206 (0.08)	-0.051 (0.15)	0.290 (0.49)
<i>Left hemisphere</i>						
Dorsolateral PFC	0.122 (0.23)	0.035 (0.13)	0.070 (0.20)	0.100 (0.15)	0.006 (0.12)	0.015 (0.27)
Ventrolateral PFC	0.058 (0.14)	-0.126 (0.17)	-0.105 (0.19)	0.032 (0.23)	-0.139 (0.14)	-0.042 (0.29)
Medial PFC	0.123 (0.20)	0.078 (0.16)	0.070 (0.12)	0.148 (0.15)	0.064 (0.16)	0.117 (0.20)
Orbitofrontal Cortex	0.112 (0.15)	0.035 (0.17)	0.045 (0.17)	0.146 (0.17)	-0.037 (0.21)	0.204 (0.41)

Table 3.3.

Mean relative concentration change of HbO ($\pm$ SD) for each region of interest during S2 production across groups and sessions

Regions of interest	Pre-test		Post-test		Retention	
	Control group	Trained group	Control group	Trained group	Control group	Trained group
<i>Right hemisphere</i>						
Dorsolateral PFC	0.045 (0.12)	0.107 (0.13)	0.067 (0.16)	0.128 (0.15)	0.002 (0.16)	0.076 (0.14)
Ventrolateral PFC	0.019 (0.21)	0.090 (0.18)	-0.092 (0.20)	0.025 (0.20)	-0.060 (0.13)	0.019 (0.20)
Medial PFC	0.091 (0.12)	0.087 (0.15)	-0.028 (0.15)	0.184 (0.19)	-0.014 (0.13)	0.139 (0.19)
Orbitofrontal Cortex	0.167 (0.15)	0.186 (0.16)	-0.122 (0.14)	0.179 (0.16)	-0.051 (0.25)	0.292 (0.33)
<i>Left hemisphere</i>						
Dorsolateral PFC	0.075 (0.13)	0.139 (0.18)	0.089 (0.21)	0.080 (0.12)	0.001 (0.16)	-0.002 (0.16)
Ventrolateral PFC	0.072 (0.16)	0.046 (0.15)	-0.021 (0.25)	-0.015 (0.12)	-0.100(0.15)	-0.016 (0.20)
Medial PFC	0.008 (0.13)	0.072 (0.17)	0.018 (0.16)	0.212 (0.21)	-0.038 (0.13)	0.040 (0.15)
Orbitofrontal Cortex	0.082 (0.15)	0.121 (0.22)	-0.020 (0.18)	0.173 (0.14)	-0.001 (0.26)	0.170 (0.31)

Table 3.4.

Mean relative concentration change of HbO ($\pm$ SD) for each region of interest during S3 production across groups and sessions

Regions of interest	Pre-test		Post-test		Retention	
	Control group	Trained group	Control group	Trained group	Control group	Trained group
<i>Right hemisphere</i>						
Dorsolateral PFC	0.030 (0.12)	0.114 (0.17)	0.065 (0.17)	0.054 (0.25)	-0.013 (0.20)	-0.058 (0.25)
Ventrolateral PFC	0.047 (0.18)	0.011 (0.18)	-0.061 (0.20)	-0.004 (0.29)	-0.069 (0.22)	-0.101 (0.15)
Medial PFC	0.007 (0.11)	0.081 (0.13)	0.061 (0.16)	0.105 (0.17)	-0.050 (0.10)	-0.101 (0.28)
Orbitofrontal Cortex	0.101 (0.16)	0.157 (0.17)	0.008 (0.18)	0.117 (0.20)	-0.064 (0.17)	-0.130 (0.28)
<i>Left hemisphere</i>						
Dorsolateral PFC	0.067 (0.12)	0.059 (0.19)	0.092 (0.22)	0.008 (0.11)	-0.007 (0.18)	-0.136 (0.27)
Ventrolateral PFC	-0.073 (0.17)	-0.061 (0.15)	-0.036 (0.22)	-0.119 (0.18)	-0.068 (0.20)	-0.219 (0.12)
Medial PFC	-0.009 (0.10)	0.052 (0.13)	0.056 (0.14)	0.078 (0.12)	-0.040 (0.08)	-0.104 (0.13)
Orbitofrontal Cortex	0.026 (0.15)	0.052 (0.23)	0.077 (0.16)	-0.005 (0.14)	-0.030 (0.13)	-0.090 (0.14)

Table 3.5.

Mean relative concentration change of HbO ($\pm$ SD) for each region of interest during S4 production across groups and sessions

Regions of interest	Pre-test		Post-test		Retention	
	Control group	Trained group	Control group	Trained group	Control group	Trained group
<i>Right hemisphere</i>						
Dorsolateral PFC	0.061 (0.18)	0.049 (0.10)	0.138 (0.22)	0.100 (0.30)	-0.018 (0.16)	0.075 (0.21)
Ventrolateral PFC	-0.002 (0.26)	-0.072 (0.19)	0.042 (0.21)	0.105 (0.28)	-0.069 (0.09)	0.083 (0.22)
Medial PFC	0.130 (0.14)	0.077 (0.16)	0.100 (0.20)	0.097 (0.21)	-0.034 (0.13)	0.014 (0.21)
Orbitofrontal Cortex	0.018 (0.21)	0.090 (0.15)	0.010 (0.11)	0.146 (0.25)	-0.062 (0.20)	0.267 (0.31)
<i>Left hemisphere</i>						
Dorsolateral PFC	0.128 (0.21)	0.012 (0.13)	0.131 (0.14)	0.030 (0.30)	-0.004 (0.17)	0.045 (0.27)
Ventrolateral PFC	-0.021 (0.24)	-0.127 (0.16)	0.021 (0.16)	-0.016 (0.28)	-0.080 (0.20)	-0.061 (0.28)
Medial PFC	0.065 (0.13)	0.007 (0.15)	0.128 (0.18)	0.053 (0.14)	-0.010 (0.11)	0.018 (0.18)
Orbitofrontal Cortex	-0.034 (0.22)	0.011 (0.15)	0.142 (0.13)	0.039 (0.23)	-0.039 (0.19)	0.165 (0.39)

4. Chapter 4

Do different motion correction techniques influence the statistical and processing outcomes of a motor sequence learning task? A functional near-infrared spectroscopy study (fNIRS)³

³ A version of this chapter is currently under revision (phase: revise and re-resubmit)

Polskaia, N., Fraser, S., & Lajoie, Y. (2021). Do different motion correction techniques influence the statistical and processing outcomes of a motor sequence learning task? A functional near-infrared spectroscopy study (fNIRS). *Neurophotronics*.

4.1. Abstract

Significance: Functional near-infrared spectroscopy (fNIRS) is susceptible to signal contaminants of various origins (e.g., instrumentation, motion artifacts and physiology). Failing to properly identify and remove signal contaminants can compromise the recovery of the hemodynamic response function (HRF). Motion correction techniques have been developed to address this issue; however, no guidelines have been established for motor sequence learning. This can have an impact on the statistical outcomes and, subsequently, the scientific conclusions.

Aim: We examined the influence of five motion correction techniques (PCA, spline, wavelet, spline + wavelet, and CBSI) on the statistical outcomes of a motor sequence learning experiment. The techniques were also compared to determine their efficacy.

Approach: Each technique was applied to cerebral oxygenation data collected during a five-day motor sequence learning experiment. To determine efficacy, the techniques were compared across two metrics: recovered HRF and within-subject standard deviation.

Results: Findings revealed that the location of significance does vary depending on the correction. No significant difference between techniques was observed for the recovered HRF. Wavelet and spline + wavelet reduced within-subject standard deviation compared to spline in a limited number of conditions.

Conclusions: Motion correction should be selected based on the dataset and type of signal contaminants.

4.2. Introduction

Functional near-infrared spectroscopy (fNIRS) is a non-invasive neuroimaging technique that uses near-infrared light (NIR) to determine changes in cerebral oxygenation (Ferrari & Quaresima, 2012; Huppert, 2016; Scholkmann et al., 2014). By capturing changes in NIR light intensity, relative concentrations of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) can be quantified (Perrey, 2008). Compared to other neuroimaging techniques, fNIRS is inexpensive, portable, provides high temporal resolution, and enables long-term evaluation (Boas, Elwell, Ferrari, & Taga, 2014; Quaresima & Ferrari, 2016). This has increased the study of cerebral oxygenation in a variety of tasks and populations (Boas et al., 2014; Scarapicchia, Brown, Mayo, & Gawryluk, 2017; Yucel, Selb, Huppert, Franceschini, & Boas, 2017).

While fNIRS presents distinct advantages, its sensitivity to signal contaminants makes recovery of the hemodynamic response function (HRF) challenging. There are three main sources of contaminants: instrumental noise (e.g., noise arising from the device and/or surrounding equipment), motion artifacts, and systemic physiology (Herold, Wiegel, Scholkmann, & Muller, 2018; Huppert, Diamon, Franceschini, & Boas, 2009). Motion artifacts can be characterized as high-frequency spikes, baseline shifts, or low-frequency changes (Brigadoi et al., 2014). In addition, they can be independent of the stimuli or synchronized with the HRF. Physiological components arise from both, extracerebral (e.g., skin, skull, cerebrospinal fluid, etc.) and cerebral tissue and exhibit specific frequency profiles (Hocke et al., 2018; Tachtsidis & Scholkmann, 2016). This includes heart rate (~ 1 Hz), respiration (0.2 – 0.4 Hz), Mayer waves (~ 0.1 Hz), and very low-frequency oscillations (skin blood flow associated with the peripheral vasculature; 0.02 – 0.08 Hz) (Kirilina et al., 2013; Pinti, Scholkmann, Hamilton, Burgess, & Tachtsidis, 2019; Yucel et al., 2016). Several spatial and temporal

methods have been developed to identify and remove contaminants from the signal (Hocke et al., 2018; Tak & Ye, 2014). This includes methods such as bandpass filter (Huppert et al., 2009; Hocke et al., 2018), principal component analysis (Zhang, Brooks, Franceschini, & Boas, 2005), wavelet filtering (Molavi & Dumont, 2012), spline interpolation (Scholkmann, Spichtig, Muehlemann, & Wolf, 2010), and correlation-based signal improvement (CBSI) (Cui, Bray, & Reiss, 2010).

However, to date, there is no standardization or agreed upon signal processing method for fNIRS (Hocke et al., 2018; Pfeifer, Scholkmann, & Labruyere, 2018; Pinti et al., 2018). Additionally, with pre-processing information sparsely reported and variability in signal processing pipelines and corresponding tuning parameters, it is difficult to determine a motion correction technique that is appropriate for a given experimental paradigm (Hocke et al., 2018; Pinti et al., 2019). Failing to properly minimize or remove signal contaminants can result in interpreting stimulus-related systemic changes as hemodynamic response-related (i.e., false-positive) or attenuating the HRF altogether (i.e., false-negative) (Tachtsidis & Scholkmann, 2016; Pfeifer et al., 2018). This can affect the statistical inferences and ultimately lead to the dissemination of faulty information (Pinti et al., 2019; Pfeifer et al., 2018; Pinti et al., 2018).

To establish signal processing guidelines for fNIRS, a comparison of motion correction techniques across various domains and populations is required, especially with the growing number of commercially available systems (Quaresima & Ferrari, 2016) and processing software (e.g., HomER2, nirsLab, NIRS-SPM). So far, only a small number of studies have compared corrections available through Homer2 (Brigadoi et al., 2014; Behrendt, Firk, Nelson, & Perdue, 2018; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al., 2015). Of the studies mentioned, processing recommendations have varied, possibly due to differences in populations (e.g.,

infants, children, stroke patients, young adults), tasks (e.g., Stroop, auditory grammatical judgement task, resting-state, face-to-face expression sequences) and degree of signal contaminants. To our knowledge, no study has evaluated the influence of signal processing methods available through HomER2 on the statistical outcome and HRF recovery of a motor sequence learning study. Therefore, the aim of this study was two-fold: to compare five motion correction techniques and their respective processing pipelines on the statistical outcomes of a motor sequence learning experiment and second, to determine which correction is most suitable to this experimental paradigm using metrics such as recovered HRF and within-subject standard deviation.

4.3. Materials and methods

The complete dataset and protocol from Polskaia, St-Amant, Fraser, and Lajoie (in review) was used for the purpose of this study.

4.3.1. Participants

Fourteen, right-handed, young adults participated in the study (24.71 ± 2.05 years). The study was approved by the University of Ottawa Ethics Committee. Exclusion criteria consisted of any self-reported neurological and/or visual impairments.

4.3.2. Functional near-infrared spectroscopy (fNIRS)

Prefrontal cortex (PFC) activation was acquired through changes in concentration of HbO using a continuous-wave spectrometer (Octamon, Artinis Medical Systems, Einsteinweg, Netherlands) with eight light emitting diodes (760 and 850 nm) and two photodiode detectors, resulting in an eight-channel system (T1 – T8). Optode distance was fixed at 35 mm. The

sampling frequency was set at 10 Hz. By using the modified 10/20 positioning system to ensure proper optode placement, it was presumed that T1/T7 were located over the dorsolateral PFC (DLPFC), T2/T8 over the ventrolateral PFC (VLPFC), T3/T5 over the medial PFC (mPFC) and T4/T6 over the orbitofrontal PFC (Polskaia, St-Amant, Fraser, & Lajoie, 2020). Since the DLPFC and VLPFC are common regions of interest (ROI) in the literature, only data collected from these regions was examined for the purpose of this study.

4.3.3. Sequences

Participants were instructed to perform four sequences (S1, S2, S3, S4) of right handed finger-tapping. Each sequence was eight units in length with 1, 2, 3, 4, 5 corresponding to the thumb, index, middle, ring, and pinky fingers, respectively. The digits were randomized for each sequence and did not include any sequential runs (i.e., 1-2-3). Participants were randomly assigned to one of two experimental groups: control or trained. For the control group, all four sequences (S1 – S4) were performed during pre-test, post-test, and retention. In addition to performing all four sequences during pre-test, post-test and retention, the trained group was required to learn two sequences (S1 and S2) across four days of practice.

4.3.4. fNIRS acquisition and practice

Cerebral oxygenation was acquired during pre-test (day 1), post-test (day 4), and retention (day 5). The acquisition sessions consisted of the four sequences (S1- S4) in an ON-OFF cycle paradigm of rest (baseline) and active blocks (experimental task). For each sequence, rest and active blocks were 20-seconds in duration and repeated four and three times, respectively. For the rest blocks, participants were instructed to relax and focus on the fixation

cross displayed on the screen. This enabled all extraneous stimuli to be accounted for. For the active blocks, participants were instructed to perform S1, S2, S3, or S4. Participants in the trained group attended 45-minute practice sessions across four consecutive days.

4.3.5. Motion correction techniques

4.3.5.1. Principal component analysis (PCA)

The principal component analysis (PCA) transforms a dataset into a series of uncorrelated components (Brigadoi et al., 2014; Zhang et al., 2005). These components are organized by their contribution of variance (Brigadoi et al., 2014; Cooper et al., 2012). The assumption is that motion artifacts make up a large percentage of the observed variance (Huppert et al., 2009; Brigadoi et al., 2014; Cooper et al., 2012). Therefore, the first eigenvectors should capture the variance associated with motion. The eigenvectors are then subtracted from the data matrix Y . In HomER2, the PCA function depends on the tuning parameter, nSV , which determines the percentage of variance to remove. Based on the findings of Brigadoi et al. (2014), a value of 0.80 was selected to remove components that account for 80% of the variance. A higher value could potentially remove a portion of the hemodynamic response function.

4.3.5.2. Wavelet

The wavelet function in HomER2 follows in part the procedure outlined by Molavi and Dumont (2012). Each signal ($y(t)$) is transformed using the discrete wavelet transform (DWT). According to Molavi and Dumont (2012), the signal is composed of the physiological signal ($f(t)$) and the interference term ($e(t)$):

$$y(t) = f(t) + e(t).$$

The function assumes that the wavelet coefficients have a Gaussian distribution (Molavi & Dumont, 2012). This is based on the premise that the hemodynamic function is relatively smooth compared to motion artifacts. As a result, the wavelet coefficients associated with the hemodynamic response should be around zero with minimal variability. Conversely, coefficients representing motion artifacts should appear as outliers (i.e., large values) (Brigadoi et al., 2014; Molavi & Dumont, 2012). To remove coefficients related to motion artifacts, a probability threshold (α) is applied. If the probability of values being larger than the coefficient is less than α , it is assumed that the coefficient represents motion and is set to zero (Molavi & Dumont, 2012). The signal is then reconstructed with the inverse discrete wavelet transform (Brigadoi et al., 2014). α (arbitrary probability) or interquartile range (iqr) in HomER2 is considered a tuning parameter. An $iqr = 0.5$ was selected based on the recommendation put forward by Di Lorenzo et al. (2019) when analyzing data involving active blocks of 10-seconds or longer.

4.3.5.3. Spline interpolation

The spline interpolation developed by Scholkmann et al. (2010) uses a channel-by-channel design to identify and correct motion artifacts. Only data points exceeding the thresholds outlined in section 4.3.6. are given non-zero values and then extracted from the signal using the HomER2 `hmrMotionArtifactbyChannel` function. Then a cubic spline interpolation function is applied to each identified motion artifact segment (Scholkmann et al., 2010). The interpolated segment is then subtracted from the initial signal revealing the denoised segment. However, the subtraction process produces different signal levels that need to be adjusted to ensure a uniform signal. Therefore, each segment is then shifted by its mean value and the mean value of the previous segment, taking into consideration the segment length (Scholkmann et al., 2010). The accuracy of the spline interpolation is determined by the tuning parameter, p , or `p_Spline` in

HomER2. A $p = 0$ applies a straight-line function, whereas $p = 1$ applies a cubic spline interpolation (Scholkmann et al., 2020). A value of $p_Spline = 0.99$ was derived from Brigadoi et al. (2014), Cooper et al. (2012), Di Lorenzo et al. (2019) and Scholkmann et al. (2010) and used for this study.

4.3.5.4. Correlation-based signal improvement (CBSI)

The correlation-based signal improvement (CBSI) method proposed by Cui et al. (2010) assumes that HbO and HbR should be negatively correlated. Therefore, if motion artifacts are present in the signal, they should be reflected as positive correlations. This produces the following equations:

$$x = x_0 + \alpha F + \text{Noise}$$

$$y = y_0 + F + \text{Noise}$$

where x and y are the measured HbO and HbR, respectively, and x_0 and y_0 are the true HbO and HbR signal, respectively. F is the signal contaminant (e.g., motion artifact), which has a similar influence on HbO and HbR and is subject to a constant factor (α). Noise is high frequency white noise originating from the instrumentation with independent effects on HbO and HbR (Cui et al., 2010). In order to calculate x_0 and y_0 , the function assumes: x_0 and y_0 should be negatively correlated (close to -1) and F and x_0 should have a correlation close to 0. This produces the following equations:

$$x_0 = (x - \alpha y)/2$$

$$y_0 = -(1/\alpha) x_0$$

where α is the ratio of the standard deviation of the measured HbO and HbR (Brigadoi et al., 2014; Cui et al., 2010). A tuning parameter is not required for executing the CBSI function in HomER2.

4.3.6. HomER2

All processing streams were performed using HomER2 (Huppert et al., 2009). Figure 4.1 outlines the processing steps for each of the six streams. For each stream, raw optical intensity data was converted into changes in optical density (OD; `hmrIntensity2OD`). Apart from the no correction stream (i.e., `bandpass`), a motion artifact detection function, `hmrMotionArtifact` or `hmrMotionArtifactbyChannel`, was applied to the OD before or after the motion correction. Parameters for these functions were derived from Brigadoi et al. (2014), Cooper et al. (2012) and Di Lorenzo et al. (2019). The algorithms identified data points as motion artifacts if the signal exceeded an amplitude of 0.4 (`AMPThresh`) or a standard deviation of 20 (`SDThresh`) over a period of 1-second (`tMotion`). Data points exceeding these parameters were masked for 1-second before and after (`tMask`). `HmrMotionArtifact` is not a channel specific function, in that it assumes that motion identified in one channel is present in all channels. As a result, `hmrMotionArtifactbyChannel` is required for the spline interpolation stream (described in greater detail in section 4.3.5.3). A fourth-order Butterworth bandpass filter was also applied to all streams to remove any additional sources of noise (e.g., physiological and instrumental noise) (`hmrBandpassFilt`) (Huppert et al., 2009). A low-pass filter of 0.5 Hz was applied to remove high-frequency instrumental noise and heart rate. A high-pass filter of 0.01 Hz was applied to remove any low-frequency drift (Cooper et al., 2012). For all streams, data was converted (`hmrOD2Conc`) from OD to relative concentration using the modified Beer-Lambert law

4.3.7. Statistical analysis

To satisfy the first objective (i.e., statistical outcome), we performed group (control, trained) x session (pre-test, post-test, retention) x sequence (S1, S2) x channel (T1, T2, T7, T8) mixed analysis of variance (ANOVA) on the relative concentration change of HbO (block average) with repeated measures on the last three factors. The HRF was block averaged to increase the signal-to-noise ratio and reliability of the fNIRS signal (Gervain et al., 2011). This analysis was performed on the relative concentration change of HbO generated by each motion correction technique. The same set of analyses were performed on the relative concentration change of HbO during the production of S3 and S4.

To satisfy the second objective (i.e., correction performance), we compared each motion correction technique by performing channel (T1, T2, T7, T8) x motion correction technique (bandpass, PCA, wavelet, spline, spline + wavelet, CBSI) repeated measures (RM) ANOVA on the relative concentration change of HbO (block average) for each group (control, trained), session (pre-test, post-test, retention) and sequence (S1, S2, S3, S4). Additionally, within-subject standard deviation was computed for each motion correction technique and analyzed with a channel (T1, T2, T7, T8) x motion correction technique (bandpass (no correction), PCA, wavelet, spline, spline + wavelet, CBSI) RM ANOVA for each session, sequence, and group.

When required, Bonferroni was used to determine the location of significance. Statistical significance was set to $p < 0.05$.

4.4. Results

4.4.1. Motion correction techniques vs. statistical results

A summary of the significant main effects and interactions for S1 and S2 for all motion correction techniques are reported in Table 4.1 and Table 4.2. All remaining effects for each correction failed to reach statistical significance ($p_s > 0.05$). A summary of the significant main effect for S3 and S4 for all corrections is reported in Table 4.3. All remaining effects for each correction failed to reach statistical significance ($p_s > 0.05$).

Table 4.1

Significant interactions on the relative concentration change of HbO ([HbO]) for sequence-1 and sequence-2 production across all motion correction techniques

Motion Correction	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparisons	
							Control group	Trained group
Bandpass	group x session x channel	6	72	2.73	0.019	0.185		
PCA				3.10	0.009	0.205		
Wavelet				2.52	0.029	0.173	↓ [HbO] in right and left VLPFC during retention vs. pre-test ($p s < 0.05$)	
Spline				3.03	0.011	0.202	↓ [HbO] in left VLPFC during post-test vs. pre-test ($p < 0.05$)	
Spline + wavelet				2.33	0.041	0.163	↑ [HbO] in right VLPFC during pre-test vs. post-test and retention ($p s < 0.05$)	
CBSI				3.78	0.003	0.239	↓ [HbO] in left VLPFC during post-test vs. pre-test ($p < 0.05$)	↑ [HbO] in left VLPFC during post-test vs. pre-test ($p < 0.05$)

Table 4.2

Significant main effects and interactions on the relative concentration change of HbO ([HbO]) for sequence-1 and sequence-2 production across all motion correction techniques

Motion Correction	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparisons
Bandpass				3.23	0.034	0.212	
PCA				4.43	0.009	0.270	↓ [HbO] in left VLPFC vs. right and left DLPFC (p s < 0.05)
Wavelet	Channel	3	36	5.57	0.003	0.317	↓ [HbO] in left VLPFC vs. right and left DLPFC (p s < 0.05)
Spline				3.18	0.036	0.209	↓ [HbO] in left VLPFC vs. right DLPFC (p = 0.055)
Spline + Wavelet				5.64	0.003	0.320	↓ [HbO] in left VLPFC vs. right and left DLPFC (p s < 0.05)
CBSI							
							Sequence 1
							Sequence 2
Bandpass							
PCA							
Wavelet	Channel x sequence	3	36	3.58	0.023	0.230	↓ [HbO] in left VLPFC vs. right DLPFC, right VLPFC, and left DLPFC (p s < 0.05)
Spline							
Spline + Wavelet	Channel x sequence	3	36	3.66	0.021	0.234	↓ [HbO] in left VLPFC vs. right DLPFC, right VLPFC, and left DLPFC (p s < 0.05)
CBSI							
Bandpass							
PCA							
Wavelet							
Spline	Session x sequence	2	24	3.41	0.050	0.221	
Spline + Wavelet							
CBSI							

Table 4.3

Significant main effect on the relative concentration change of HbO ([HbO]) for sequence-3 and sequence-4 production across all motion correction techniques

Motion Correction	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparisons
Bandpass	Channel	3	36	5.02	0.005	0.295	↓ [HbO] in left VLPFC vs. left DLPFC ($p < 0.05$)
PCA				5.93	0.002	0.331	↓ [HbO] in left VLPFC vs. left DLPFC ($p < 0.05$)
Wavelet				6.82	0.001	0.362	↓ [HbO] in left VLPFC vs. right DLPFC, right VLPFC and left DLPFC ($p \leq 0.05$)
Spline				4.96	0.006	0.292	↓ [HbO] in left VLPFC vs. left DLPFC ($p < 0.05$)
Spline + wavelet				7.84	0.0004	0.395	↓ [HbO] in left VLPFC vs. right DLPFC, right VLPFC and left DLPFC ($p \leq 0.05$)
CBSI							

4.4.2. Comparison of motion corrections across group, session, and sequence

4.4.2.1. Trained group

Figure 2 (D – F) provides an example of the recovered mean HRFs during S1 production for the right hemisphere (T1 and T2) across all corrections for the trained group.

For S1, all main effects and filter x channel interactions failed to reach statistical significance for pre-test and post-test ($p_s > 0.05$; $\eta p^2 = 0.118 - 0.470$). For retention, the ANOVA revealed a significant filter x channel interaction, $F(15, 90) = 2.103$, $p < 0.05$, $\eta p^2 = 0.260$. The post-hoc analysis revealed no significant pairwise comparisons.

For S2, all main effects and filter x channel interactions failed to reach statistical significance for pre-test, post-test, and retention ($p_s > 0.05$, $\eta p^2 = 0.061 - 0.314$).

For S3, the main effect of channel, $F(3, 18) = 5.70$, $p < 0.05$, $\eta p^2 = 0.487$, was superseded by a filter x channel interaction $F(15, 90) = 5.27$, $p < 0.0001$, $\eta p^2 = 0.468$, for pre-test. The post-hoc analysis revealed no significant pairwise comparisons. All main effects and filter x channel interaction failed to reach statistical significance for post-test ($p_s > 0.05$, $\eta p^2 = 0.109 - 0.223$). For retention, the ANOVA revealed a significant filter x channel interaction, $F(15, 90) = 3.057$, $p < 0.01$, $\eta p^2 = 0.338$. The post-hoc analysis revealed significantly less cortical activity in the left VLPFC with the wavelet filter ($M = -0.219$) compared to the CBSI ($M = -0.012$) ($p = 0.041$).

For S4, the main effect of channel, $F(3, 18) = 4.35$, $p < 0.05$, $\eta p^2 = 0.420$, was superseded by a filter x channel interaction, $F(15, 90) = 2.53$, $p < 0.01$, $\eta p^2 = 0.297$, for pre-test. The post-hoc analysis revealed no significant pairwise comparisons. All main effects and filter x

channel interactions failed to reach statistical significance for post-test and retention ($p_s > 0.05$, $\eta p^2 = 0.048 - 0.216$).

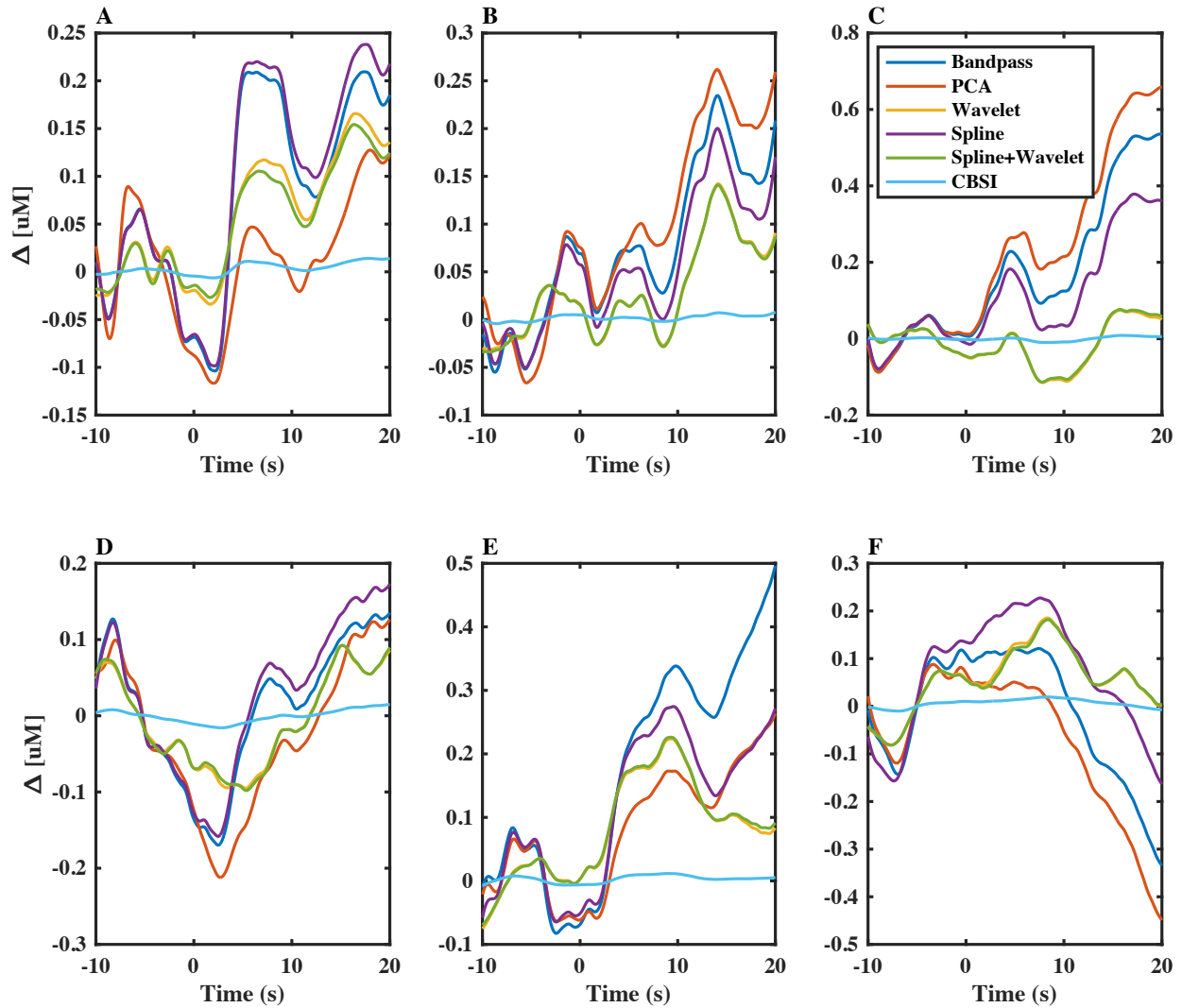


Figure 4.2. Recovered mean hemodynamic response functions (HRF) for the right hemisphere (dorsolateral PFC (DLPFC) and ventrolateral PFC (VLPFC) for all motion correct techniques including bandpass (no correction) during sequence-1 production. **(A)** pre-test, control group; **(B)** post-test, control group; **(C)** retention, control group; **(D)** pre-test, trained group; **(E)** post-test, trained group; **(F)** retention, trained group. Each colour represents a different motion correction technique.

4.4.2.2. Control group

Figure 2 (A – C) provides an example of the recovered mean HRFs during S1 production for the right hemisphere (T1 and T2) across all corrections for the control group.

For S1, all main effects and filter x channel interaction failed to reach statistical significance for pre-test ($p_s < 0.05$, $\eta p^2 = 0.051 - 0.237$). For post-test, the main effect of channel, $F(3, 18) = 4.50$, $p < 0.05$, $\eta p^2 = 0.454$, was superseded by a filter x channel interaction, $F(15, 90) = 4.66$, $p < 0.0001$, $\eta p^2 = 0.437$. The post-hoc analysis revealed no significant pairwise comparisons. For retention, the main effect of channel, $F(3, 18) = 5.76$, $p < 0.05$, $\eta p^2 = 0.490$, was superseded by a filter x channel interaction, $F(15, 90) = 3.32$, $p < 0.0001$, $\eta p^2 = 0.356$. The post-hoc analysis revealed no significant pairwise comparisons.

For S2, all main effects and filter x channel interaction failed to reach statistical significance for pre-test ($p_s > 0.05$, $\eta p^2 = 0.036 - 0.218$). For post-test, the main effect of channel, $F(3, 18) = 4.69$, $p < 0.05$, $\eta p^2 = 0.439$, was superseded by a filter x channel interaction, $F(15, 90) = 2.95$, $p < 0.01$, $\eta p^2 = 0.329$. The post-hoc analysis revealed no significant pairwise comparisons. For retention, the ANOVA revealed a significant filter x channel interaction, $F(15, 90) = 1.92$, $p < 0.05$, $\eta p^2 = 0.242$. The post-hoc analysis revealed no significant pairwise comparisons.

For S3, all main effects and filter x channel interaction failed to reach statistical significance for pre-test ($p_s > 0.05$, $\eta p^2 = 0.175 - 0.202$). For post-test, the main effect of channel, $F(3, 18) = 7.72$, $p < 0.01$, $\eta p^2 = 0.563$, was superseded by a filter x channel interaction, $F(15, 90) = 5.63$, $p < 0.001$, $\eta p^2 = 0.484$. The post-hoc analysis revealed no significant pairwise comparisons. For retention, all main effects and filter x channel interaction failed to reach statistical significance ($p_s > 0.05$, $\eta p^2 = 0.124 - 0.225$).

For S4, all main effects and filter x channel interactions failed to reach statistical significance for pre-test, post-test, and retention ($p_s > 0.05$, $\eta p^2 = 0.022 - 0.251$).

4.4.3. Motion correction vs. standard deviation across group, session, and sequence

A summary of the significant main effects of filter for the trained and control groups across sessions and sequences are reported in Table 4.4 and Table 4.5, respectively. A summary of the significant filter x channel interactions across sessions and sequences for both groups is reported in Table 4.6. The main effect of channel failed to reach statistical significance across all sessions, sequences, and groups ($p_s > 0.05$).

Table 4.4.

Significant main effect of filter on within-subject standard deviation (SD) across session and sequence for the trained group only

Group	Sequence	Session	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparisons
Trained	S1	Pre-test	Filter	5	30	23.14	0.0005	0.794	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)
		Post-test				8.28	0.007	0.580	↓ SD with CBSI compared to wavelet, spline and spline + wavelet ($p s < 0.05$)
		Retention				9.25	0.011	0.607	↓ SD with CBSI compared to wavelet, spline and spline + wavelet ($p s < 0.05$)
	S2	Pre-test				19.86	0.001	0.768	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)
		Post-test				22.63	0.0005	0.790	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)
		Retention				14.65	0.007	0.709	↓ SD with CBSI compared to PCA, wavelet, and spline + wavelet ($p s < 0.05$)
	S3	Pre-test				20.30	0.001	0.778	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)
		Post-test				13.29	0.005	0.689	↓ SD with CBSI compared to PCA and spline + wavelet ($p s < 0.05$)
		Retention				63.49	< 0.0001	0.914	↑ SD with bandpass compared to PCA, spline + wavelet and CBSI ($p s < 0.05$)
	S4	Pre-test				24.02	< 0.0001	0.800	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)
		Post-test				7.73	0.017	0.563	↓ SD with CBSI compared to bandpass, wavelet, spline, and spline + wavelet ($p s < 0.05$)
		Retention				24.91	< 0.0001	0.806	↓ SD with CBSI compared to the remaining filters ($p s < 0.05$)

Table 4.5.

Significant main effect of filter on within-subject standard deviation (SD) across session and sequence for the control group only

Group	Sequence	Session	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparisons
Control	S1	Pre-test	Filter	5	30	37.94	< 0.0001	0.863	↑ SD with bandpass compared to wavelet, spline + wavelet, and CBSI ($ps < 0.05$) ↓ SD with wavelet compared to spline ($p < 0.05$) ↓ SD with spline + wavelet compared to spline ($p < 0.05$) ↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Post-test				6.43	0.043	0.517	↓ SD with CBSI compared to wavelet and spline + wavelet ($ps < 0.05$)
		Retention				11.92	0.010	0.665	↓ SD with CBSI compared to wavelet, spline and spline + wavelet ($ps < 0.05$)
	S2	Pre-test				26.67	< 0.001	0.816	↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Post-test				29.88	< 0.0001	0.833	↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Retention				28.81	0.0010	0.828	↓ SD with wavelet compared to spline ($p < 0.05$) ↓ SD with spline + wavelet compared to spline ($p < 0.05$) ↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
	S3	Pre-test				52.76	< 0.0001	0.898	↓ SD with PCA compared to bandpass ($p < 0.05$) ↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Post-test				36.64	< 0.0001	0.859	↑ SD with bandpass compared to wavelet, spline + wavelet, and CBSI ($ps < 0.05$) ↓ SD with wavelet compared to spline ($p < 0.05$) ↑ SD with spline compared to wavelet, spline + wavelet and CBSI ($ps < 0.05$) ↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Retention				27.80	< 0.001	0.822	↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
	S4	Pre-test				8.44	0.019	0.584	↓ SD with CBSI compared to wavelet, spline and spline + wavelet ($ps < 0.05$)
		Post-test				29.93	< 0.001	0.833	↓ SD with wavelet compared to spline ($p < 0.05$) ↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)
		Retention				36.11	< 0.0001	0.857	↓ SD with CBSI compared to the remaining filters ($ps < 0.05$)

Table 4.6.

Significant filter x channel interaction on within-subject standard deviation (SD) across session, sequence, and group

Group	Sequence/Session	Effect	df	df_{error}	F	p	η_p^2	Pairwise comparison
Control	S2 Post-test				2.66	0.002	0.307	↓ SD with CBSI compared to bandpass, PCA and spline for the left VLPFC (T8) ($p s < 0.05$) ↓ SD with CBSI compared to all the remaining filters for right (T1) and left DLPFC (T8) ($p s < 0.05$)
Trained	S2 Post-test				4.56	< 0.001	0.432	↓ SD with CBSI compared to wavelet and spline + wavelet for the right DLPFC (T1) ($p s < 0.05$) ↓ SD with CBSI compared to all the remaining filters for the right VLPFC (T2) ($p s < 0.05$) ↓ SD with wavelet compared bandpass and spline for the left DLPFC (T7) ($p s < 0.05$) ↓ SD with spline + wavelet compared to bandpass and spline for the left DLPFC (T7) ($p s < 0.05$) ↓ SD with CBSI compared to all the remaining filters for the left DLPFC (T7) ($p s < 0.05$) ↓ SD with CBSI compared to all the remaining filters for the left VLPFC (T8) ($p s < 0.05$)
		filter x channel	15	90				
	S3 Retention				3.73	< 0.0001	0.384	↓ SD with wavelet compared to bandpass and PCA for the right VLPV (T2)

4.5. Discussion

The objective of the present study was two-fold: first, to examine the influence of different motion correction techniques on the statistical outcome of a motor sequence learning paradigm and second, to compare the performance of these techniques to promote standardization of fNIRS in motor sequence learning research. Our findings revealed that different motion correction techniques do in fact lead to different statistical outcomes when applied to the same dataset. Interestingly, no significant differences were observed between corrections in the recovered HRF. Additionally, wavelet and spline + wavelet improved within-subject standard deviation compared to spline but in a limited number of conditions.

Motion correction vs. statistical results

The results in Table 4.1 demonstrate that the location of significance will vary depending on the motion correction technique selected. Each method apart from bandpass and PCA, resulted in statistically different interactions between groups, channels, and sessions for sequence-1 and 2. Therefore, conclusions drawn from each correction will inevitably differ. For instance, with significance observed between pre-test and retention, wavelet, and spline + wavelet enable inferences that can potentially differentiate cortical activation associated with learning from that of performance. Similar interpretations cannot be made with spline or CBSI processed data, as significance was limited to pre-test and post-test. Additionally, while all corrections generated significance in the VLPFC region, hemispheric contributions (right (T2) vs left (T8)) varied across corrections. Only wavelet yielded significant changes in both, the right and left VLPFC channels. As well, comparison between groups would only be permitted with

CBSI, as all remaining corrections had outcomes limited to the control group. Any interferences made with regards to the trained group would solely be based on visual observations.

Our findings also revealed similarities in statistical outcomes across several correction methods (Table 4.2). PCA, wavelet and spline + wavelet led to significant differences between the left VLPFC and both hemispheric regions of the DLPFC. Likewise, wavelet and spline + wavelet revealed significant differences between the left VLPFC and all remaining channels during S1 production. The similarity in corrections, particularly between the PCA and spline + wavelet, may be a result of poor motion artifact detection (Brigadoi et al., 2014; Hu et al., 2015), which is discussed in greater detail below.

Statistical similarity between corrections was also observed for S3 and S4 production data (Table 4.3). Bandpass, PCA and spline led to significant differences between the left VLPFC and left DLPFC channels. Whereas wavelet and spline + wavelet led to significance between the left VLPFC and all remaining channels. These findings demonstrate the need for processing guidelines to ensure that accurate interpretations of hemodynamic response data are made.

Comparison metric: Recovered hemodynamic response function (HRF)

Our findings revealed no significant difference between corrections when compared across the recovered HRF for all four sequences. Figure 4.2 demonstrates that all corrections, apart from CBSI, performed comparably across sessions and groups. This contrasts previous studies that have reported improved performance with spline, wavelet, targeted PCA (tPCA) and spline + wavelet (Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al.,

2014). Our conflicting results may be a consequence of poor motion artifact detection (Brigadoi et al., 2014) and experimental paradigm.

Unlike previous studies (Cooper et al., 2012; Scholkmann et al., 2010), spline interpolation did not perform well with our dataset. The recovered HRF was very similar in shape to the no motion correction (i.e., bandpass) HRF (Figure 4.2). We suspect that the motion artifacts were not properly identified with the `hmrMotionArtifactbyChannel` function due to their similarity in frequency, amplitude, and possible synchronization with the HRF (Brigadoi et al., 2014). As a result, the spline interpolation was not applied, and these periods of contaminated data were only bandpass filtered. Similarly, Di Lorenzo et al. (2019) found spline to have the worst performance in recovering the true HRF, as evidenced by a high root-mean-square-error (RMSE) value. In addition, our findings suggest that the parameters outlined in section 4.3.6 for the `hmrMotionArtifactbyChannel` function may not be suitable for a motor sequence learning paradigm and will require refinement moving forward. A similar suggestion was put forward by Di Lorenzo et al. (2019) when applying the spline + wavelet technique, which too requires the use of the `hmrMotionArtifactbyChannel` function.

The wavelet filter recovered HRFs that were also comparable with the other techniques, including the no correction (Figure 4.2). Like spline, we suggest that our motion artifacts were similar in profile to the HRF, and at times, the wavelet coefficients were not properly assigned (Brigadoi et al., 2014; Molavi & Dumont, 2012). More specifically, the function may have attenuated the HRF by setting the coefficients of those data points to zero (Brigadoi et al., 2014). Generally, the wavelet technique is most effective at identifying and removing high-frequency motion artifacts (Molavi & Dumont, 2012; Hu et al., 2015), although, it has been found to be effective in removing motion artifacts of lower frequency (Brigadoi et al., 2014). Brigadoi et al.

(2014) found wavelet filtering to be the best at reducing trial rejection, standard deviation (e.g., within and between) and area under the curve. The inconsistency between our findings and Brigadoi et al. (2014) may be due to the dataset and experimental paradigm (Stroop task vs. finger-tapping task, respectively).

In addition, the combination of the two aforementioned techniques yielded no significant difference. Figure 4.2 clearly demonstrates that the spline + wavelet technique recovered HRFs similar in shape to the wavelet correction. As previously mentioned, we suspect that motion artifacts were not properly detected with the `hmrMotionArtifactbyChannel` function (Brigadoi et al., 2014) and as a result, only the wavelet correction was performed. Conversely, spline + wavelet has been shown to be an optimal correction method for infant datasets as evidenced by the reduction in within- and between-subject standard deviation (Di Lorenzo et al., 2019). Since adults can perform a finger-tapping task with minimal movement, it is possible that combining the corrections (spline + wavelet) may not be more effective at removing lower frequency motion artifacts.

Our data revealed that the PCA correction did not differ in performance from the other techniques, including the no correction method (Figure 4.2). Previous studies have also reported minimal improvements with PCA on metrics such as trial rejection, area under the curve, standard deviation, HRF and mean-squared error when compared to other motion corrections (Brigadoi et al., 2014; Cooper et al., 2012). We suggest that our poor performance resulted from a failure to satisfy certain PCA requirements (Brigadoi et al., 2014; Cooper et al., 2014; Hu et al., 2015; Virtanen, Noponen, & Merilainen, 2009). PCA is a method that requires motion artifacts to be present in several channels (Brigadoi et al., 2014; Cooper et al., 2012; Hu et al., 2015). It is possible that due to the placement of the optodes, certain artifacts were not detected by all

channels. As a result, this may have caused components of the signal unrelated to motion to be removed (Cooper et al., 2012). Additionally, the expectation that extracerebral and cerebral components of the signal are independent was most likely not met by our protocol (Virtanen et al., 2009; Zhou, Sobczak, McKay, & Litovsky, 2020). We suspect that certain contaminants were correlated to the HRF (Franceschini, Fantini, Toronov, & Gratton, 2000; Scholkmann et al., 2014), which would have made it difficult for the PCA to effectively identify and remove the variance associated with the extracerebral response. Finger-tapping has been found to precipitate a correlation between the stimulation frequency and several physiological responses such as heart rate, respiration, and arterial pulse (Franceschini et al., 2000).

Lastly, the CBSI correction method did not significantly differ from the other corrections in the recovered HRF. Visually, the CBSI-recovered HRFs are very different in shape than the remaining techniques (Figure 4.2). We suspect that our data contained periods of positive correlation between HbO and HbR that were not motion artifact related (Brigadoi et al., 2014). It is possible that in response to cortical activation, an increase in cerebral blood flow (CBF) precipitated a simultaneous increase in HbR (Fujiwara et al., 2004; Murata, Sakatani, Katayama, & Fukaya, 2002). As a result, components of the hemodynamic response were filtered along with the motion artifacts, ultimately contributing to the over filtered signal. Our findings do contradict the results of Brigadoi et al. (2014) and Hu et al. (2015) that have demonstrated improved performance using CBSI across several metrics such as area under curve, within-subject and between-subject standard deviation. The inconsistency in results may be a consequence of our experimental paradigms (e.g., Stroop task, auditory grammatical judgement language task, and finger tapping, respectively). Studies have found motor and working memory tasks to affect the

fNIRS signal by provoking systemic changes at the extracerebral and cerebral level (e.g., blood pressure) (Scholkmann et al., 2014).

Comparison metric: Within-subject standard deviation (SD)

Our data revealed that the motion correction techniques differed when evaluated using the within-subject standard deviation (SD) metric (Table 4.4 and Table 4.5). The CBSI correction method significantly reduced the within-subject SD in comparison to the other techniques. This was observed across groups, sessions, and sequences. However, this was partly to be expected as the concentration values were extremely small with CBSI as opposed to the other corrections (Figure 4.3). As previously mentioned, the low values may indicate that in addition to the removal of noise, components of the hemodynamic response were filtered out as well. Interestingly, all remaining findings apart from one were limited to the control group (Table 4.5). This trend in our findings cannot be readily explained.

We found increased SD with bandpass (i.e., no motion correction) compared to wavelet, spline + wavelet and CBSI. However, this significance was limited to S1 pre-test and S3 post-test for the control group and S3 retention for the trained group (Figure 4.3). Similar observations were found by Brigadoi et al. (2014) and Di Lorenzo et al. (2019) when compared against the no correction method. All remaining sessions and sequences had increased SD with bandpass but were unable to reach statistical significance. Additionally, our findings revealed reduced SD with wavelet and spline + wavelet when compared to spline alone. However, this was limited to S1 pre-test, S2 retention and S3 post-test (Figure 4.3). Since the reduced SD using wavelet and spline + wavelet only emerged in certain sessions, sequences, and channels (Table

4.6), further examination is required to determine if the wavelet filter is a suitable pre-processing method for motor sequence learning data.

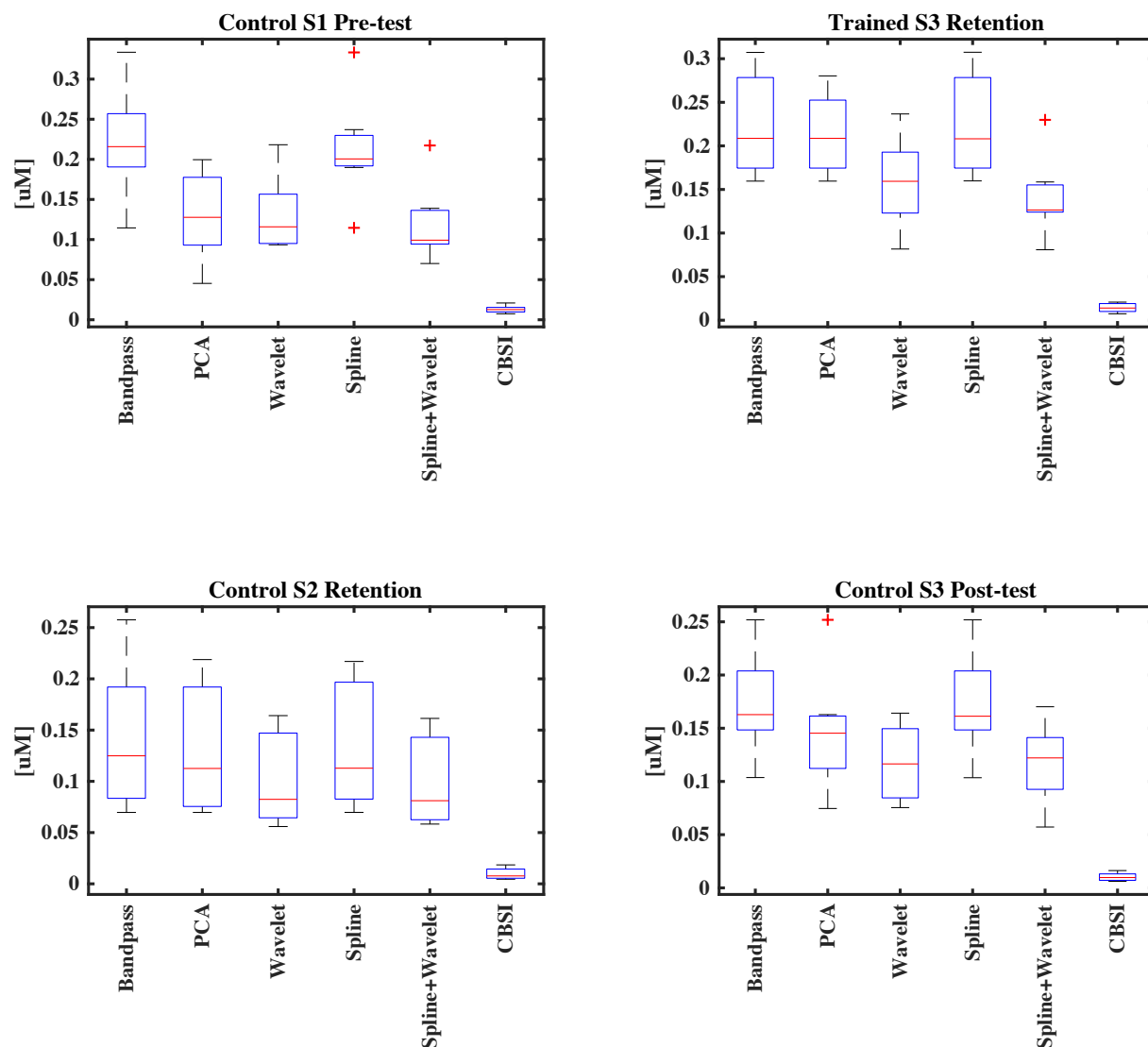


Figure 4.3. Box-plots of within-subject standard deviation (SD) for all motion correction techniques including bandpass (no correction) across certain sessions and sequences. The red line represents the median value, and the whiskers represent the first and third quartile. The red cross represents outliers.

Tuning parameters

Our findings highlight the importance of selecting appropriate tuning parameters for functions such as `hmrMotionArtifact`, `hmrMotionArtifactbyChannel`, `bandpass`, `wavelet` and `PCA` when applying them to a finger-tapping task. This is a critical step for ensuring signal contaminants are properly identified and removed, while components of the hemodynamic response function are retained.

Our findings suggest that `hmrMotionArtifact` and `hmrMotionArtifactbyChannel` are not effective in detecting motion artifacts that are comparable in frequency and amplitude to the HRF. Therefore, tuning the amplitude and standard deviation threshold values may only be advantageous if the signal is predominately contaminated by high frequency spike artifacts (Hu et al., 2015). Otherwise, if thresholds selected are too low (e.g., `AMPThresh` = 0.1), the function may end up attenuating task-related components in the signal. Furthermore, we also suggest that motion correction techniques that require prior motion detection may not be effective for finger-tapping tasks that contain low frequency motion artifacts.

In terms of bandpass filtering, the cut-off frequency parameters have ranged across studies, with 0.01 – 0.5 Hz being the most commonly used (Pinti et al., 2019). However, this range, does not remove signal contaminants such as respiration and blood pressure, as they overlap in frequency with the hemodynamic response (Huppert et al., 2009). Recently, Pinti et al. (2019) put forward the recommendation that cut-off frequencies could be determined according to the design's stimulation frequency. In particular, the low-pass could be set to 0.09 Hz, as long as the stimulation frequency falls below said value. This would preserve the stimulation frequency, while simultaneously filtering out respiration (~0.3 Hz) and Mayer waves (~0.09 Hz). Nonetheless, this recommendation and its suitability for motor sequence learning paradigms requires further investigation.

In our study, an iqr value of 0.5 was effective in reducing within-subject SD in certain conditions and channels but did not recover HRFs that were significantly different from the remaining corrections. In the literature, iqr values have ranged from 0.1 to 1.5 (Brigadoi et al., 2014; Behrendt et al., 2018; Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al., 2015; Molavi & Dumont, 2012) but according to Behrendt et al. (2018) and Di Lorenzo et al. (2019), an iqr value of 0.5 is suitable for blocked designs (10-seconds or more) and provides a compromise between removing noise from the signal and potentially underestimating the HRF.

In the literature, nSV values for PCA have ranged from 0.80 – 0.97 (Brigadoi et al., 2014; Cooper et al., 2012; Hu et al., 2015; Wilcox, Bortfeld, Woods, Wruck, & Boas, 2005). Presently, selecting an nSV value of 0.80 (i.e., removing 80% of the total variance) did not recover HRFs that were significantly different from the remaining motion corrections and the no correction method. We suspect that due to the lack of independence between the cerebral and extracerebral hemodynamics, removing 81% or more of the variance would not have yielded superior results (Brigadoi et al., 2014; Virtanen et al., 2009). Most likely, it would have removed components of the hemodynamic response. Additionally, PCA may not be a suitable approach for motor learning tasks that can cause a correlation between the extracerebral and cerebral responses.

4.6. Conclusion

In conclusion, our findings revealed that statistical inferences can vary depending on the motion correction technique selected. As well, these techniques may not be effective at identifying and removing motion artifacts that are low frequency, low amplitude and potentially correlated with the HRF, which we suspect to be common in motor sequence learning tasks.

With limited improvement in standard deviation, further research is required to determine if wavelet filtering is the best approach. In addition, corrections that require prior motion detection (e.g., spline) or an independence between extracerebral and cerebral activity (e.g., PCA) may not be appropriate for motor sequence learning paradigms.

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5. Chapter 5

Neural correlates of dual-task processing following motor sequence learning: A functional near-infrared spectroscopy (fNIRS) study⁴

⁴ A version of this chapter will be submitted for publication.

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5.1. Abstract

Dual-task paradigms are used to evaluate movement automaticity by comparing the performance of two tasks when executed simultaneously. The neural correlates of dual-task processing, before and after practice, remains unclear. The current study used functional near-infrared spectroscopy (fNIRS) to examine cerebral oxygenation changes in the prefrontal cortex (PFC) associated with dual-task processing before and after motor sequence practice.

Participants performed self-initiated sequential finger movements of varying complexity (4 and 12 units) with a visual letter-counting task. After practice, dual-task sequence-4 performance revealed decreased activity in the right dorsolateral PFC, medial PFC, and orbitofrontal cortex. A similar but marginal trend was observed in the right VLPFC between pre-test and post-test.

Conversely, dual-task sequence-12 performance revealed increased activity in the right ventrolateral PFC. The findings suggest that dual-task interference was reduced following practice for dual-task sequence-4. The results also suggest that increased right hemisphere activation is needed to maintain performance when the primary sequential task (e.g., dual-task sequence-12) has a high level of difficulty.

5.2. Introduction

Dual-task paradigms are commonly used to evaluate movement automaticity by comparing the performance of two tasks executed simultaneously. The primary task is considered automatic when it can be performed with minimal to no interference from the secondary task (Passingham, 1996; Salings & Phillips, 2007). Generally, dual-task interference is high in early learning when attentional resources are limited and decreases once the primary task is well established (Remy, Wenderoth, Lipkens, & Swinnen, 2010). The neural correlates underlying dual-task processing, especially before and after learning, have not been thoroughly researched (Erickson et al., 2006; Poldrack et al., 2005; Remy et al., 2010).

Conversely, the neural correlates associated with motor sequence learning have been extensively studied, revealing cortical involvement of structures such as the prefrontal cortex (PFC), the primary motor cortex (M1), the premotor cortex (PMC), supplementary motor area (SMA), cerebellum, and basal ganglia (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Doyon et al., 2002; Eliassen, Souza, & Sanes, 2001; Grafton, Hazeltine, & Ivry, 1995; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Jueptner et al., 1997; Karni et al., 1995; Lehericy et al., 2005; Penhune & Doyon, 2002; Steele & Penhune, 2010; Toni, Krams, Turner, & Passingham, 1998; Wu, Kansaku, & Hallett, 2004). Specific to the PFC, neuroimaging studies have shown decreased activation following practice, suggesting minimal cortical involvement once the skill has been learned (Jenkins et al., 1994; Jueptner et al., 1997; Wu et al., 2004). At the same time, a subset of neuroimaging studies has also shown increased PFC activation following learning (Doyon et al., 1996; Doyon et al., 2002; Eliassen et al., 2001; Grafton et al., 1995; Hazeltine, Grafton, & Ivry, 1997; Honda et al., 1998; Penhune & Doyon, 2002; Polskaia, St-Amant, Fraser, & Lajoie, 2020; Steele & Penhune, 2010). This is suggested to reflect the

explicit learning and working memory requirements of the motor sequence task. Interestingly, only a few studies have implemented dual-task paradigms to determine if the automatic phase of motor learning was achieved following practice (Poldrack et al., 2005; Polskaia et al., 2020; Wu et al., 2004; Wu, Liu, Hallett, Zheng, & Chan, 2013). This is necessary because improvements to behaviour can be observed without achieving automaticity (Lang & Bastian, 2002; Polskaia et al., 2020).

Additionally, to our knowledge, only a handful of studies have evaluated the neural correlates of dual-task processing during the early and late phase of motor sequence learning (Poldrack et al., 2005; Wu & Hallett, 2005; Wu, Liu, Hallett, Zheng, & Chan, 2013). Poldrack et al. (2005) found practice to be associated with decreased activity in the ventral premotor cortex, inferior frontal gyrus, dorsolateral prefrontal cortex (DLPFC), and right caudate region. The authors cited several possible reasons for the observed findings, including cognitive control, retrieval of stimulus-response maps, task switching, and response-selection conflicts. Similarly, Wu & Hallett (2005) reported reduced activity in several cortical regions, including the left DLPFC during dual-task performance. Also, in addition to reduced activation in the pre-SMA, premotor cortex, and right postcentral gyrus following practice, Wu et al. (2013) revealed increased functional connectivity between the cerebellum and regions such as the pre-SMA, premotor cortex, M1, temporal gyrus, and thalamus. To contribute to this work, our current study evaluates cerebral oxygenation changes in the PFC associated with dual-task processing before and after motor sequence learning using functional near-infrared spectroscopy (fNIRS). fNIRS is a non-invasive neuroimaging technique that can quantify the relative concentration of oxyhemoglobin (HbO), deoxyhemoglobin (HbR), and total hemoglobin (tHb) by capturing changes in light intensity in the near-infrared range (700 – 900 nanometers (nm)) (Bunce,

Izzetoglu, Onaral, & Pourrezaei, 2006; Scholkmann et al., 2014). It offers portability, higher temporal resolution, and greater tolerance to motion artifacts than fMRI (Perrey, 2008).

Based on the increase in right hemisphere activation observed during explicit motor sequence learning (Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998) and in our single-task motor sequence learning study (Polskaia et al., 2020), we hypothesized that contrary to Poldrack et al. (2005) and Wu & Hallett (2005), dual-task activity in the post-test session would be represented by increased activity in the right dorsolateral and ventrolateral PFC. We suspected this increase would reflect explicit learning and retrieval of information from long-term memory.

5.3. **Materials and methods**

5.3.1. Participants

Seventeen, right-handed, young adults participated in the study (23.25 ± 2.21 years). The study was approved by the University of Ottawa Ethics Committee. Exclusion criteria consisted of any self-reported neurological and/or visual impairments.

5.3.2. Functional near-infrared spectroscopy (fNIRS)

PFC activation was acquired through task-related changes in the relative concentration of HbO and HbR using a continuous-wave spectrometer (Octamon, Artinis Medical Systems, Einsteinweg, Netherlands). The device was equipped with eight light emitting diodes (760 and 850 nm) and two photodiode detectors, resulting in an eight-channel system (T1-T8). Channels T1-T4 were situated over the right hemisphere, and channels T5-T8 were situated over the left hemisphere. Optode distance was fixed at 35 mm. The sampling frequency was set at 10 Hz. By

using the modified 10/20 positioning system to ensure proper optode placement, it was presumed that T1/T7 were located over the dorsolateral PFC (DLPFC), T2/T8 over the ventrolateral PFC (VLPFC), T3/T5 over the medial PFC (mPFC) and T4/T6 over the orbitofrontal cortex.

5.3.3. Additional apparatus

A four-button response device was used to record participants' sequence production (e.g., number of key presses and frequency of responding) (Figure 5.1). All key presses were transmitted to a computer equipped with National Instrument data acquisition software. A 13" monitor secured above the participant's head was used to provide the visual prompts.

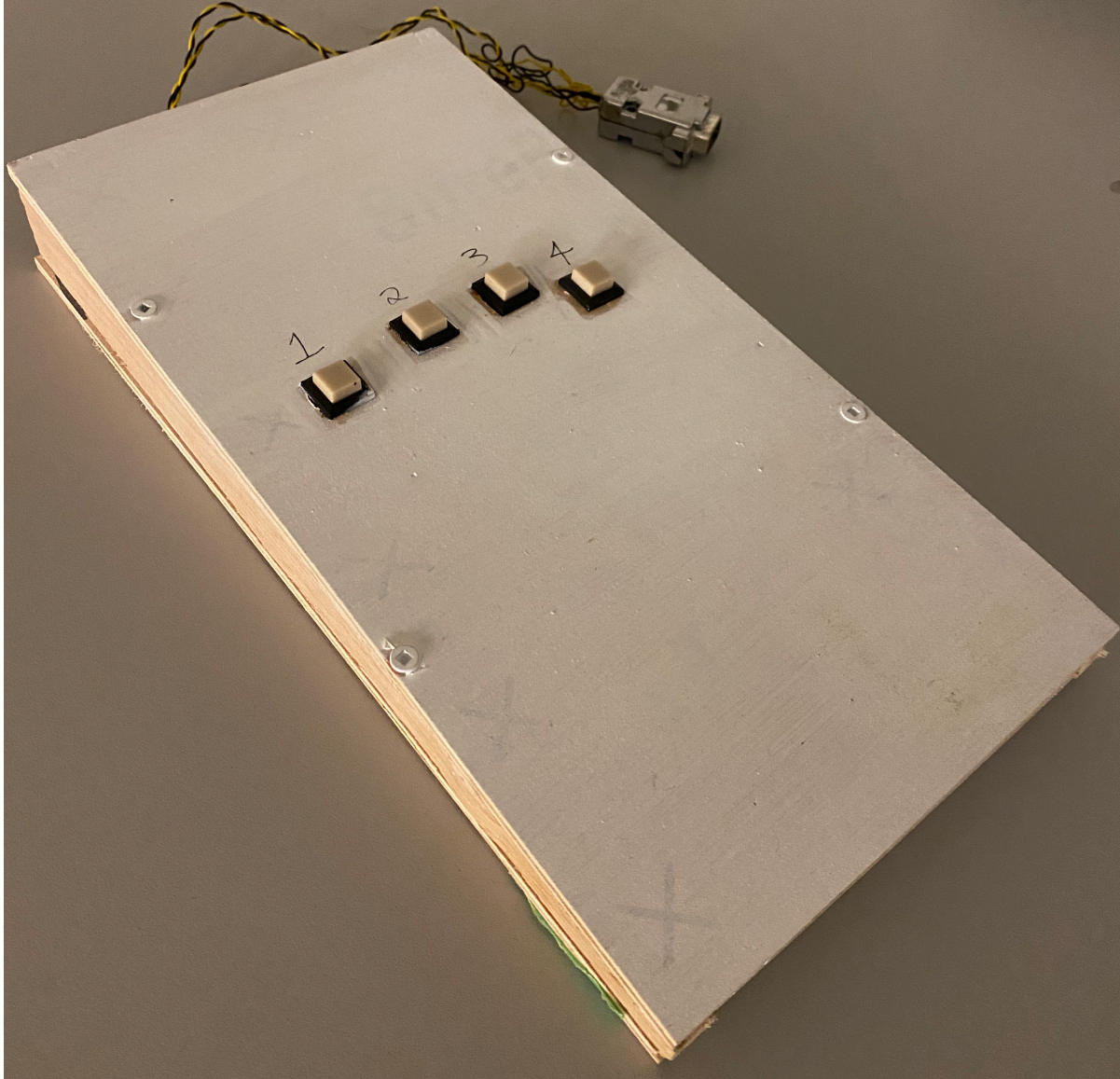


Figure 5.1. Four-button response device. 1, 2, 3, and 4 correspond to the index, middle, ring, and pinky fingers, respectively.

5.3.4. Dual-task

Following the protocol of Wu et al. (2004), participants were instructed to perform two sequences of right hand finger-tapping while concurrently performing a visual letter-counting task. The sequences were classified according to the number of units they were comprised of.

Sequence-4 (S4) consisted of 1-3-4-2, in which 1, 2, 3, and 4 corresponded to the index, middle, ring, and pinky fingers, respectively. Sequence-12 (S12) consisted of 1-4-3-2-2-4-1-3-4-1-2-3. To control for speed, participants were instructed to perform the sequences at 0.5 ± 0.1 Hz. At the required speed, S4 was completed 4 times and S12 was completed 1 time during the active block (25-seconds). The visual letter-counting task consisted of a random sequence of A, G, L, and O, and participants were required to silently count the total number of times the target letter (e.g., A) was presented. As stipulated by Wu et al. (2004), all movements were self-initiated, and the speed was not driven by an external cue.

5.3.5. fNIRS acquisition

fNIRS was acquired during pre-test and post-test. The acquisition involved the concurrent performance of the sequential task (S4 or S12) and the visual letter-counting task in an ON-OFF cycle paradigm of rest (baseline) and active (experimental task). Prior to acquisition, participants received a brief familiarization period (approximately 10-20 minutes) to consistently replicate the required speed. For each dual-task (sequence + visual letter), rest and active blocks were 25-seconds in duration and were repeated six and five times, respectively. For the rest blocks, participants were instructed to relax and focus on the fixation cross displayed on the screen. For the active blocks, participants were instructed to perform dual-task S4 or dual-task S12 once the visual prompt appeared on the screen. Participants were prompted to provide their letter counts 10-seconds after the active block was completed. Feedback on performance was not provided during the acquisition sessions. All conditions were completed in a supine position to replicate similar fMRI conditions.

After pre-test, participants were allotted 2-4 hours of practice to learn S4 and S12. During practice, participants were required to complete a minimum of 2 sets of 12 trials (25-seconds each) for each sequence. After each set, participants received summary knowledge of results, which was comprised of the number of trials with errors and the number of trials that exceed the required speed. Practice continued until participants could perform S4 and S12 from memory 10 times in a row without error, as well as the dual-tasks. If an error was committed during the 10 trials, participants were required to perform another two sets of 12 practice trials. If an error was committed during the dual-tasks, participants were required to start at the beginning by completing at least two sets of 12 practice trials, followed by 10 trials in a row with no error.

5.3.6. Behavioural data

Performance on each task was recorded. For the sequential task, an incorrect key press was considered as an error. For the visual letter-counting task, errors were classified as the difference between the correct total and the total provided by the participant.

5.3.7. Data processing

Data processing was performed using HomER2 (Huppert, Diamond, Franceschini, & Boas, 2009). First, raw optical intensity data was converted into changes in optical density (OD; `hmrIntensity2OD`). Then a wavelet filter (`hmrMotionCorrectWavelet`) with an interquartile range (iqr) of 0.5 was applied (Di Lorenzo et al., 2019). Any remaining uncorrected motion artifacts were then identified and removed using a motion artifact detection algorithm (`hmrMotionArtifact`). Parameters for this function were derived from Brigadoi et al. (2014), Cooper et al. (2012) and Di Lorenzo et al. (2019). The algorithm identified data points as motion

artifacts if the signal exceed an amplitude of 0.4 (AMPThresh) or a standard deviation of 20 (SDThresh) over a period of 1-second (tMotion). Data points exceeding theses parameters were masked for 1-second before and after (tMask). HmrMotionArtifact is not a channel specific algorithm, in that it assumes that motion artifacts identified in one channel should be removed from all channels. Next, a fourth-order Butterworth bandpass filter was applied to remove any additional sources of noise (e.g., physiological and instrumental) (hmrBandpassFilt). A high-pass filter of 0.01 Hz was applied to remove any low-frequency drift (Cooper et al., 2012). A low-pass filter of 0.09 Hz was applied to remove respiration and Mayer waves, while preserving the stimulation frequency ($1/50\text{s} = 0.02\text{ Hz}$) (Pinti, Scholkmann, Hamilton, Burgess, & Tachtsidis, 2019). The filtered data was then converted from optical density to relative concentration using the modified Beer-Lambert law (Villringer & Chance, 1997; hmrOD2Conc). Lastly, a block average (hmrBlockavg) over a time range of -10-seconds and 25-seconds was calculated. The baseline of the average was set to zero by subtracting the mean of $t < 0$ from $t > 0$.

5.3.8. Dependent variables and statistical analysis

We performed a session (pre-test, post-test) x hemisphere (right, left) x channel (T1/T7, T2/T8, T3/T5, T4/T6) RM ANOVA on the relative concentration of HbO (block average) and HbR for dual-task sequence-4 and dual-task sequence-12. When required, Bonferroni was used to determine the location of significance. Statistical significance was set to $p < 0.05$. Behavioural data was analyzed using the Wilcoxon signed-rank test.

5.4. Results

5.4.1. Behavioural data

Findings revealed improved performance after practice for dual-task S4 and dual-task S12 ($Z = -2.535, p < 0.05$ and $Z = -3.186, p < 0.05$, respectively) (Table 5.1).

Table 5.1.

Performance (number of errors) of sequential finger movements and visual letter-counting task before and after practice

Task	Errors	
	Before Practice	After Practice
Sequence-4 and letter counting	$0.66 \pm 1.18 / 2.35 \pm 1.41$	$0.01 \pm 0.05 / 1.59 \pm 1.58$
Sequence-12 and letter counting	$1.67 \pm 1.82 / 5.53 \pm 2.83$	$0.29 \pm 0.55 / 1.76 \pm 1.79$

Note. Values are mean $\pm$ SD for the number of errors committed across the five active blocks.

For sequences, errors were classified as incorrect finger movements. For the letter-counting task, errors were classified as the difference between the correct total and the total provided by the participant.

5.4.2. Dual-task sequence-4

5.4.2.1. Relative concentration change of oxyhemoglobin (HbO)

The main effect of session, $F(1, 16) = 5.80, p = 0.028, \eta^2 = 0.266$, and session x hemisphere interaction, $F(1, 16) = 10.70, p = 0.005, \eta^2 = 0.401$, were superseded by a session x hemisphere x channel interaction, $F(3, 48) = 3.62, p = 0.019, \eta^2 = 0.185$. The post-hoc analysis revealed reduced cortical activity in the right DLPFC, mPFC, and orbitofrontal cortex during post-test compared to pre-test ($p_s < 0.05$) (Figure 5.2) A similar decrease in cortical activity was

observed in the right VLPFC, however, the post-hoc analysis revealed a marginal trend towards significance ($p = 0.07$). No significance was observed in the left hemisphere ($p_s > 0.05$). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

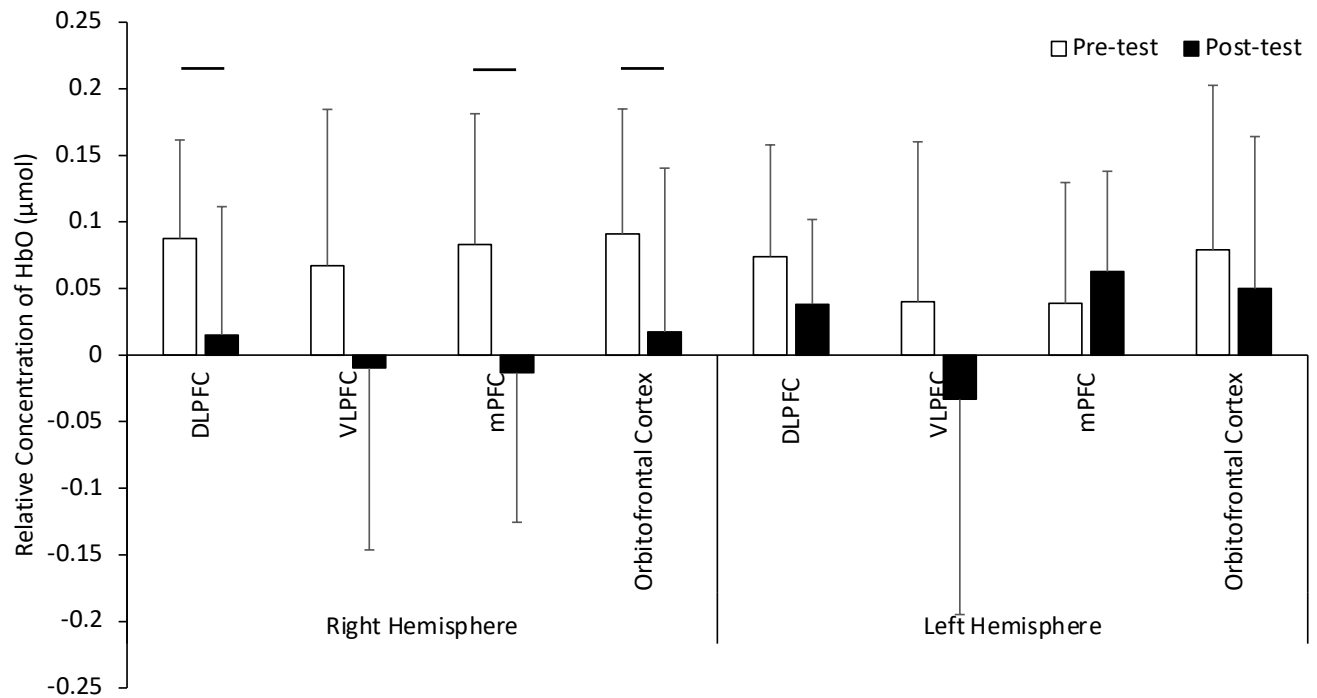


Figure 5.2. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for dual-task sequence-4 as a function of region and hemisphere. Horizontal line represents statistical significance ($p < 0.05$).

5.4.2.2. Relative concentration change of deoxyhemoglobin (HbR)

The main effect of session, $F(1, 16) = 8.96$, $p = 0.009$, $\eta^2 = 0.359$, was superseded by a session $\times$ hemisphere interaction, $F(1, 16) = 12.38$, $p = 0.003$, $\eta^2 = 0.436$. The post-hoc analysis revealed reduced cortical activity in the right hemisphere during post-test compared to

pre-test ($p < 0.001$). (Figure 5.3) No significance was observed in the left hemisphere ($p_s > 0.05$). The main effect of channel, $F(3, 48) = 4.25, p = 0.010, \eta^2 = 0.210$, was statistically significant. The post-hoc analysis revealed reduced cortical activity in the mPFC, and orbitofrontal cortex compared to DLPFC ($p_s < 0.05$). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

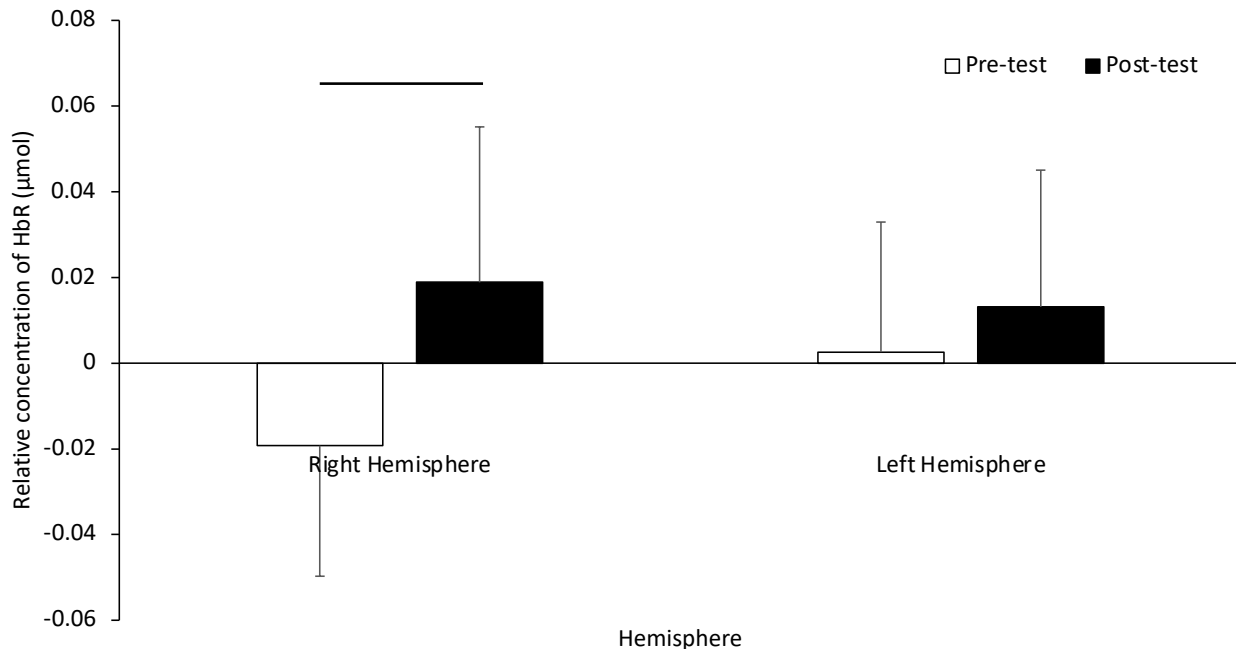


Figure 5.3. Change in relative concentration of HbR (mean $\pm$ SD) during pre-test and post-test for dual-task sequence-4 as a function of hemisphere. Horizontal line represents statistical significance ($p < 0.001$).

5.4.3. Dual-task sequence-12

5.4.3.1. Relative concentration change of HbO

The ANOVA revealed a significant hemisphere $\times$ channel interaction, $F(3, 48) = 4.72, p = 0.006, \eta^2 = 0.228$. The post-hoc analysis showed increased activity in the right VLPFC

compared to the left VLPFC ($p < 0.05$) (Figure 5.4). All remaining main effects and interactions failed to reach statistical significance ($p_s > 0.05$).

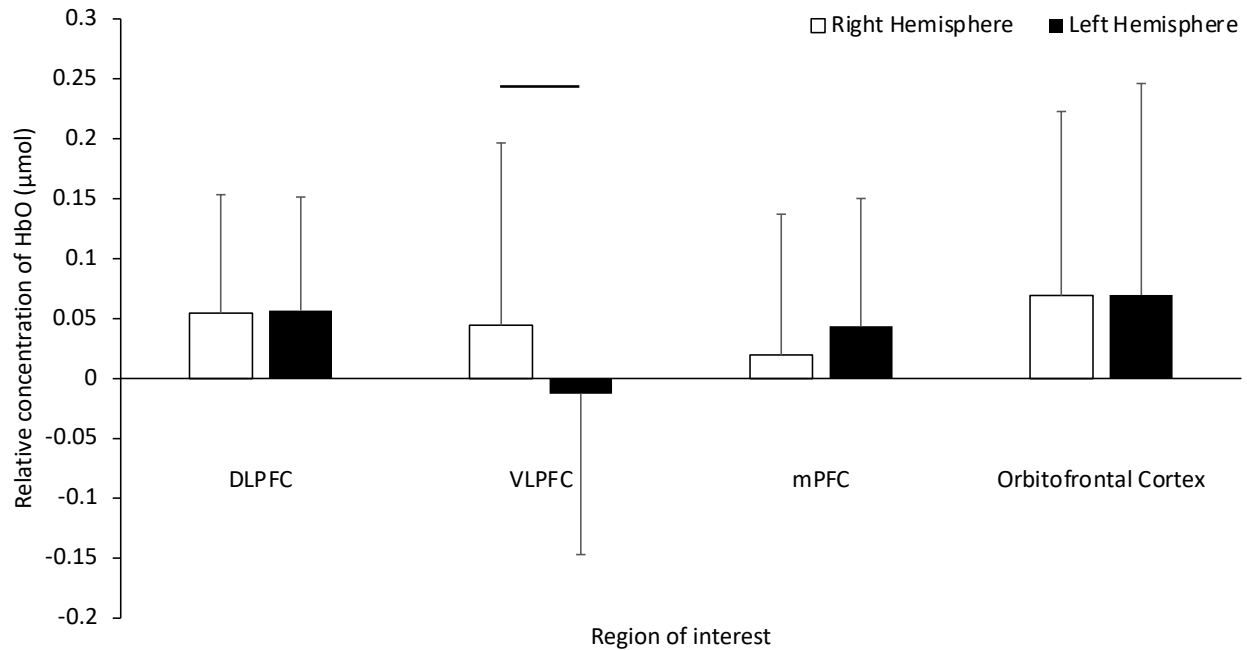


Figure 5.4. Change in relative concentration of HbO (mean $\pm$ SD) in the right and left hemisphere of the prefrontal cortex (PFC) during dual-task sequence-12 as a function of region. Horizontal line represents statistical significance ($p < 0.05$).

5.4.3.2. Relative concentration change of HbR

All main effects and interactions failed to reach statistical significance ($p_s < 0.05$).

5.5. Discussion

In the present study, we evaluated changes in PFC activation associated with dual-task processing before and after motor sequence learning. Contrary to our hypothesis, dual-task performance showed decreased activity in the right DLPFC, mPFC, and orbitofrontal cortex during post-test compared to pre-test for S4. A similar pattern was found in the right VLPFC;

however, the significance was marginal. HbR revealed corresponding findings with increased concentration in the right hemisphere in post-test compared to pre-test. Dual-task sequence-12 performance showed increased activity in the right VLPFC compared to the left. No significant changes were observed for HbR.

Behavioural performance

Our findings revealed a reduction in error score after practice for dual-task sequence-4 and sequence-12 (Table 5.1). This is consistent with several dual-task learning studies showing improved response time and accuracy with practice (Passingham, 1996; Poldrack et al., 2005; Remy et al., 2010). This suggests that practice reduced the dual-task interference observed in pre-test for both sequences. However, with errors still committed in post-test, we suggest that movement automaticity was not achieved. As stated in our single-task study (Polskaia et al., 2020), 2 to 4 hours of practice was not sufficient for ensuring movement automaticity.

Cortical data

We found decreased activity in the right DLPFC and VLPFC in post-test compared to pre-test for dual-task sequence-4 (Figure 5.2), but as previously mentioned the latter revealed a marginal trend. Therefore, the interpretations made regarding the VLPFC should be interpreted with caution. No significant differences in activation were observed in the left hemisphere for dual-task sequence-4. Our findings are consistent with several neuroimaging studies, which suggest that the DLPFC and VLPFC are necessary for processing concurrent tasks in early learning but decrease in activation when the cortical processes become more adept at managing dual-tasks (Erickson et al., 2006; Poldrack et al., 2005). In addition, increased activity in the

right VLPFC has been found to be significantly correlated to dual-task interference (Herath, Klinberg, Young, Amunts, & Roland, 2001). This would also explain why greater activation was observed during the pre-test, as both tasks required significant attention. Additionally, the lateral PFC (DLPFC and VLPFC) has been implicated in resolving response selection conflicts (Erickson et al., 2005; Poldrack et al., 2005; Schumacher & D'Esposito, 2002). Therefore, the decrease in activation from pre- to post-test may also suggest that the response selection conflicts were minimized after S4 practice. The lower error score in the post-test would further support this claim.

Previous neuroimaging studies have reported increased right hemisphere activation following single-task motor sequence learning (Doyon et al., 1996; Doyon et al., 2002; Grafton et al., 1995; Hazeltine et al., 1997; Penhune & Doyon, 2002; Polskaia et al., 2020). The previous studies have attributed these findings to explicit sequence learning and working memory processes. At this point, we can only speculate as to why the opposite was observed in the present study. We suggest that the working memory processes recruited during single-task S4 performance (Polskaia et al., 2020) were not fully necessary to maintain dual-task performance in post-test (Remy et al., 2010). We suspect this is due to the motor task's low level of difficulty. To satisfy this theory, it would then be expected that the dual-task S12 condition exhibit greater activation due to the increased complexity of the finger tapping task. This was somewhat observed in the present experiment with increased activation in the right VLPFC compared to the left VLPFC (Figure 5.3). Although the right VLPFC exhibited greater activation in post-test, the main effect of session failed to reach statistical significance (Figure 5.5). Interestingly, the right DLPFC exhibited a similar activation in post-test, which we speculate may suggest that a greater

need for response monitoring was required to ensure the improved performance was maintained (Petrides, 1996).

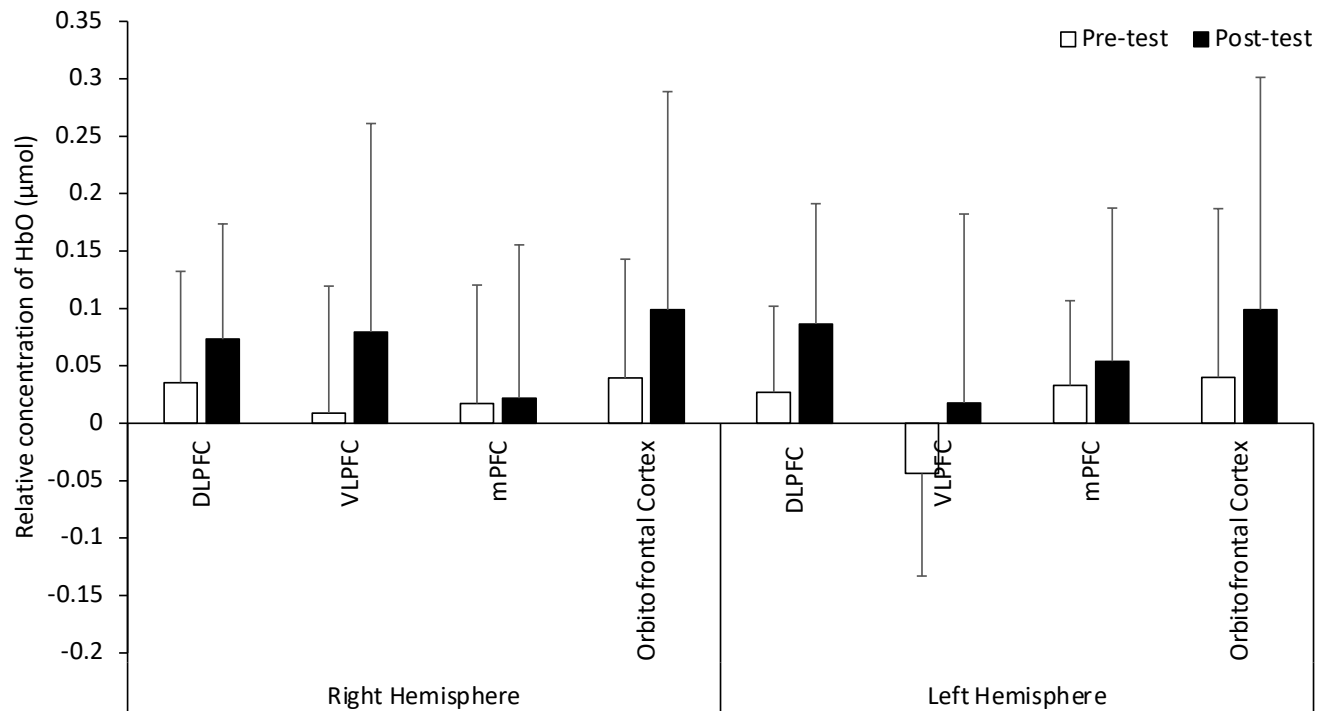


Figure 5.5. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for dual-task sequence-12 as a function of region and hemisphere.

Our findings also revealed decreased activity in the right orbitofrontal cortex in post-test compared to pre-test for dual-task sequence-4. Correspondingly, HbR revealed reduced activation in the orbitofrontal cortex. This contradicts several neuroimaging studies that have shown increased activation of the orbitofrontal cortex following motor sequence practice (Jackson, Lafleur, Malouin, Richards & Doyon, 2003; Lafleur et al., 2002; Penhune & Doyon, 2002). Interestingly, a similar increase in activation was observed in our single-task study for S4

(Polskaia et al., 2020), although significance was not found between sessions. The difference in our present findings may relate to the concurrent performance of the visual letter-counting task. We suspect that greater attention was placed on the visual task in post-test, and as a result, did not require significant activation of the orbitofrontal cortex. Additionally, the medial orbitofrontal cortex has been suggested to be involved in selecting a stimuli and/or response according to a correctness or reward value (Elliott, Dolan, & Frith, 2000). The visual letter-counting task likely provided minimal intrinsic reward, and therefore, did not require significant activation of the orbitofrontal cortex. Conversely, dual-task sequence-12 revealed increased activation of the orbitofrontal cortex in the post-test, however, the comparison failed to reach significance. It is possible that the challenge of the motor task still produced responses with great intrinsic reward. At this time, these are working hypotheses that will require further examination.

Additionally, our findings revealed reduced activation of the mPFC in post-test for dual-task sequence-4. Correspondingly, HbR revealed reduced activity in the mPFC. In addition, minimal activation was observed between sessions for dual-task sequence-12. A previous neuroimaging study has linked the mPFC to error processing (Menon, Adelman, Glover & Reiss, 2001). Although speculative, reduced activation of the mPFC may suggest that participants were not engaging in error processing during dual-task sequence-4 post-test. The decrease in error scores in post-test may further support this claim. Interestingly, the HbR findings revealed no significant differences for dual-task S12. This may be related to the sensitivity of HbR, as previous studies have shown HbO to be more sensitive to task-related hemodynamic changes (Holper et al. 2012; Miyai et al., 2001; Suzuki et al., 2004).

5.6. Conclusion

In conclusion, we observed decreased activation in the right DLPFC, mPFC, and orbitofrontal cortex in post-test for dual-task sequence-4, as a result of motor sequence practice. A decrease was also observed in the right VLPFC; however, the significance was marginal. We suspect that these changes in activity represent a reduction in dual-task interference, response selection conflicts, error processing and reward value. The improvement in behaviour may further support these claims. Conversely, increased activity in the right VLPFC was observed during dual-task sequence-12. We suspect that due to the increased complexity of the dual-task sequence-12 task, greater reliance was placed on the PFC to maintain task performance.

5.7. References

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6. General discussion

Motor sequence learning is supported by a complex and distributed network of cortical regions, which includes the prefrontal cortex (PFC), the primary motor cortex (M1), supplementary motor area (SMA), parietal cortex, cerebellum, and basal ganglia (Dahms et al., 2019). The contributions of these cortical regions have been found to differ depending on the stage of motor learning (see Lohse, Wadden, Boyd, & Hodges, 2014 for a review). Activation of the PFC, in particular, has been observed during the early/fast and late/slow phase of learning (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Doyon et al., 2002; Eliassen, Souza, & Sanes, 2001; Grafton, Hazeltine, & Ivry, 1995; Hazeltine, Grafton, & Ivry, 1997; Jenkins et al., 1994; Jueptner et al., 1997; Penhune & Doyon, 2002; Poldrack et al., 2005; Toni, Krams, Turner, & Passingham, 1998; Wu, Kansaku, & Hallett, 2004). As a result, neuroimaging studies have attributed PFC activation to processes such as movement automaticity, movement preparation, stimulus-response conflict, spatial processing, explicit learning, and memory retrieval. While previous neuroimaging studies have relied heavily on fMRI and PET, fNIRS can expand and potentially further our understanding of the PFC by providing a technique that is low in cost, portable, and has a higher temporal resolution (Perrey, 2008; Scholkmann et al., 2014). The caveat, however, is the large availability of correction methods and a current lack of standardized signal pre-processing methods for fNIRS (Hocke et al., 2018; Pinti et al., 2018). Therefore, the goal of this dissertation was to examine the capability and limitations of fNIRS in elucidating the role of the PFC in motor sequence learning and movement automaticity.

Chapter 2 of this dissertation focused on validating fNIRS as a suitable technique to fMRI by replicating a previous motor sequence learning experiment (Wu et al., 2004). The results were unable to detect the decrease in the left dorsolateral PFC (DLPFC) following

practice. However, the experiment did highlight the importance of distinguishing learning across more extended periods of time in order to better assess movement automaticity. The experiment in Chapter 3 introduced four practice sessions, a retention test, a control group, and multiple sequences to further investigate the contribution of the PFC to motor sequence learning. The findings revealed increased contribution from the right hemisphere (e.g., ventrolateral PFC (VLPFC)) and the suggestion that activation of the left DLPFC may not reflect movement automaticity but rather attentional investment in movement preparation. The experiment reported in Chapter 4 investigated the effect of five motion correction techniques on the statistical outcome of a motor sequence learning experiment. Additionally, the experiment investigated the performance of each correction across comparison metrics such as hemodynamic response function (HRF) recovery and within-subject standard deviation. The findings revealed that the location of significance does vary depending on the motion correction technique selected. Additionally, wavelet and wavelet + spline demonstrated some promise in reducing within-subject standard deviation. The experiment in Chapter 5 investigated changes in the PFC associated with dual-task processing before and after motor sequence learning. In contrast to Chapter 2, decreased activation was observed across all four regions of the right PFC (DLPFC, VLPFC (marginal trend), medial PFC, and orbitofrontal cortex), following motor sequence practice.

6.1. Movement automaticity following practice

As described at the outset of this dissertation, motor sequence learning is generally understood to follow three distinct phases of learning that can be identified by behavioural changes and specific cortical contributions (Penhune & Steele, 2012). The third phase, described

as the automatization phase, was of specific interest to this dissertation. During this phase, the motor sequence task is performed with minimal to no attentional resources directed towards the movement (Bernstein, 1967). From a neural perspective, the automatization phase has been associated with decreased activity in regions such as the DLPFC, parietal cortex, premotor cortex, and cerebellum (Poldrack et al., 2005; Wu et al., 2004), suggesting that extended practice promotes neural efficiency (see Saling & Phillips, 2007 for a review). In Chapter 2, we were focused on detecting this pattern, specifically in the left DLPFC (Wu et al., 2004), in an effort to demonstrate that fNIRS is a suitable neuroimaging technique that could detect similar automaticity-related activations as fMRI. Our findings did not reveal a significant decrease in the relative concentration of HbO ([HbO]) in the left DLPFC following practice for sequence-4 (S4) or sequence-12 (S12). Similarly, no significant difference was observed in the relative concentration of deoxyhemoglobin ([HbR]). In fact, the left DLPFC exhibited comparable activations (e.g., [HbO]) between pre-test and post-test for both sequences (Figure 6.1 and 6.2). We suspect that participants were not afforded enough practice time to achieve movement automaticity and exhibit the associated neural efficiency. Most likely, the activation patterns observed in this experiment reflect the early learning phase, where motor sequences are acquired quickly (Dahms, Brodoehl, Witte, & Klingner, 2019). Interestingly, the motor task did appear to be resistant to interference during the dual-task portion of practice, which according to the paradigm, would suggest that the motor task was automatic (Kahneman, 1973). However, based on our cortical data, we suggest that practice reduced the attentional demand enough not to exceed the resource capacity but not enough for the task to become automatic.

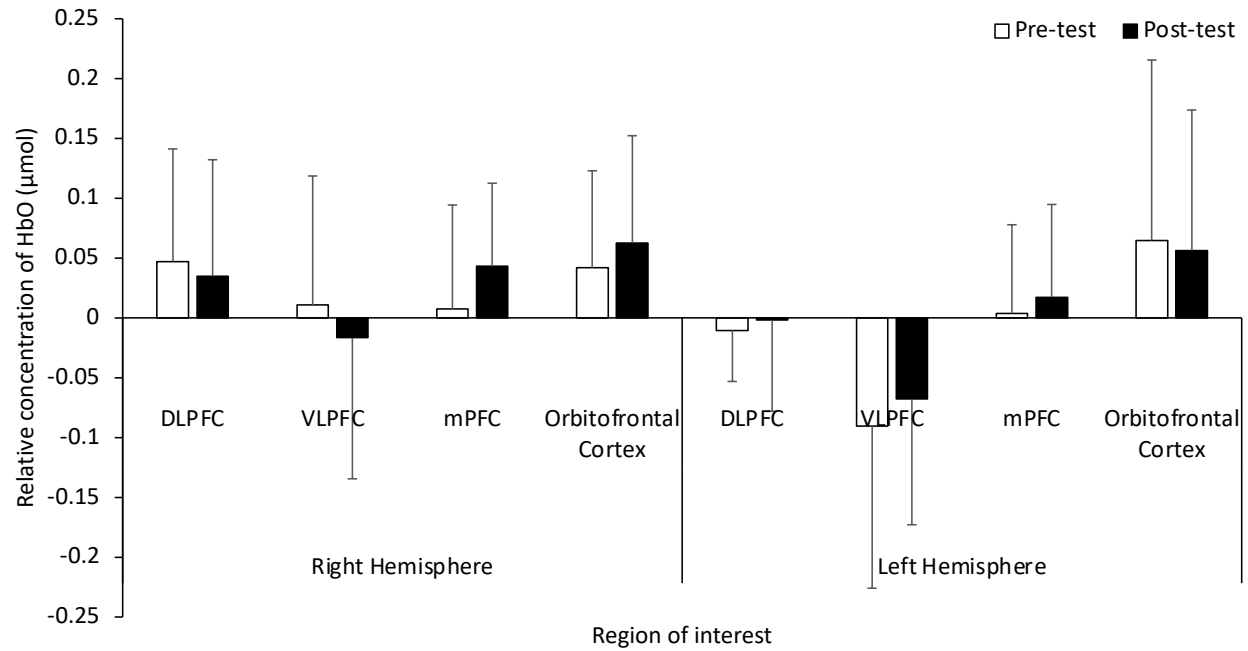


Figure 6.1. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for sequence-4 as a function of region and hemisphere (Chapter 2).

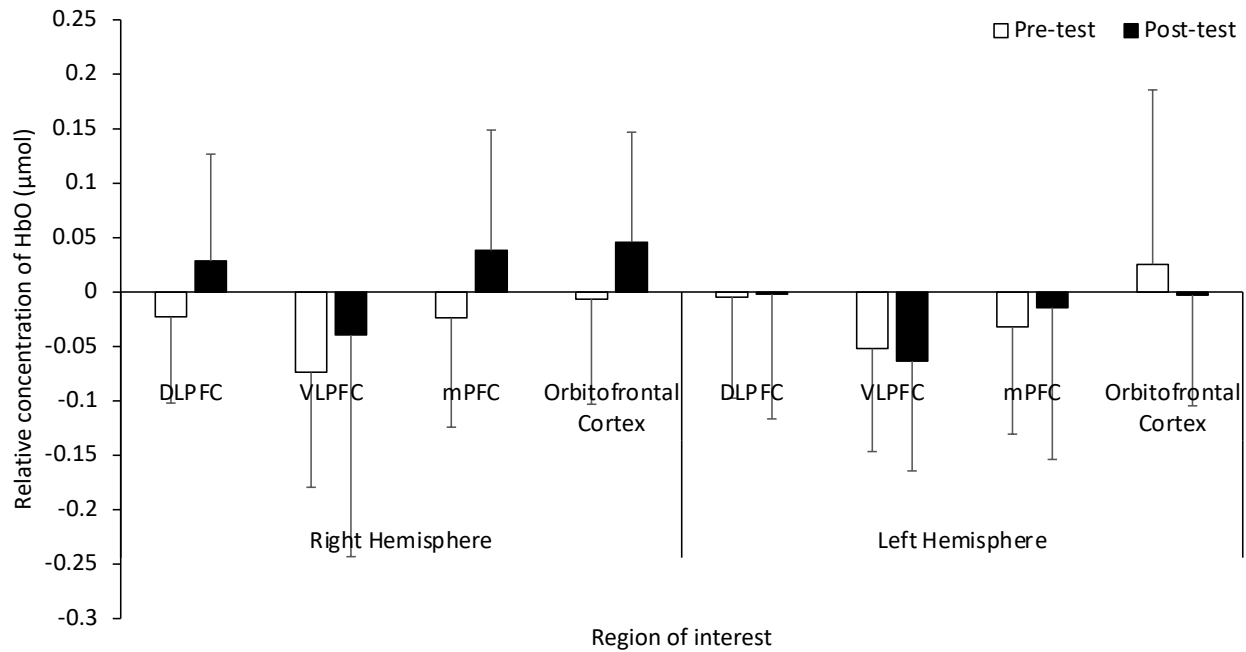


Figure 6.2. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for sequence-12 as a function of region and hemisphere (Chapter 2).

In addition, the behavioural data revealed a decrease in error scores in the post-test compared to the pre-test (Table 2.1, pg. 41). While Wu et al. (2004) associated a similar decline to automaticity, we suggest this change reflects the early phase of learning, where performance improvements are known to occur rapidly (Penhune & Steele, 2012).

Chapter 3 expanded on the findings from Chapter 2 by implementing a more classic motor learning paradigm with a control group, greater number of practice sessions, multiple sequences, and a retention test to better assess movement automaticity. Similar to Chapter 2, results showed no significant difference in activation in the left DLPFC between sessions (pre-test, post-test, and retention) for the control and trained groups for sequence-1 (S1) and sequence-2 (S2) (refer to supplementary Table 3.2. pg. 92). Contrary to previous neuroimaging

studies (Jueptner et al., 1997; Poldrack et al., 2005; Wu et al., 2004), we argue that the present findings may suggest an alternative role for the DLPFC. With activation between groups being comparable in retention, the DLPFC may not reflect movement automaticity as previously thought, but rather attentional investment in movement preparation of a pre-learned sequence (Jueptner et al., 1997). This interpretation comes from the work of Jueptner et al. (1997), where reactivation of the left DLPFC was observed only when participants were instructed to direct attention to an upcoming movement in a pre-learned sequence. Conversely, in the absence of attention, activation of the left DLPFC was not observed. This interpretation may explain why the control group exhibited similar activation as the trained group in retention (refer to Supplementary Table 3.2., pg. 92). The control group with no sequential knowledge would have been unaware of the upcoming movement and, therefore, unable to direct attention towards movement preparation. Conversely, the trained group would have been able to successfully perform the sequences without attentional investment or preparation, as a result of the extended practice. However, this experiment cannot definitively state if movement automaticity was achieved, seeing as the trained group yielded similar performance scores in pre-test and retention (Table 3.1., pg. 75). We suspect the familiarization period prior to pre-test contributed to this finding, a point that will be discussed in the limitations section.

Chapter 5 investigated the neural correlates of dual-task processing before and after motor sequence practice. Results showed no significant difference in left hemisphere activity between pre-test and post-test for dual-task sequence-4 and dual-task sequence-12. However, it is still worth discussing as the left DLPFC yielded opposing activation patterns for dual-task sequence-4 and dual-task sequence-12. Specifically, the left DLPFC exhibited decreased activity

in post-test for dual-task sequence-4 (Figure 5.2., pg. 145), and increased activity in post-test for dual-task sequence-12 (Figure 5.5., pg. 150).

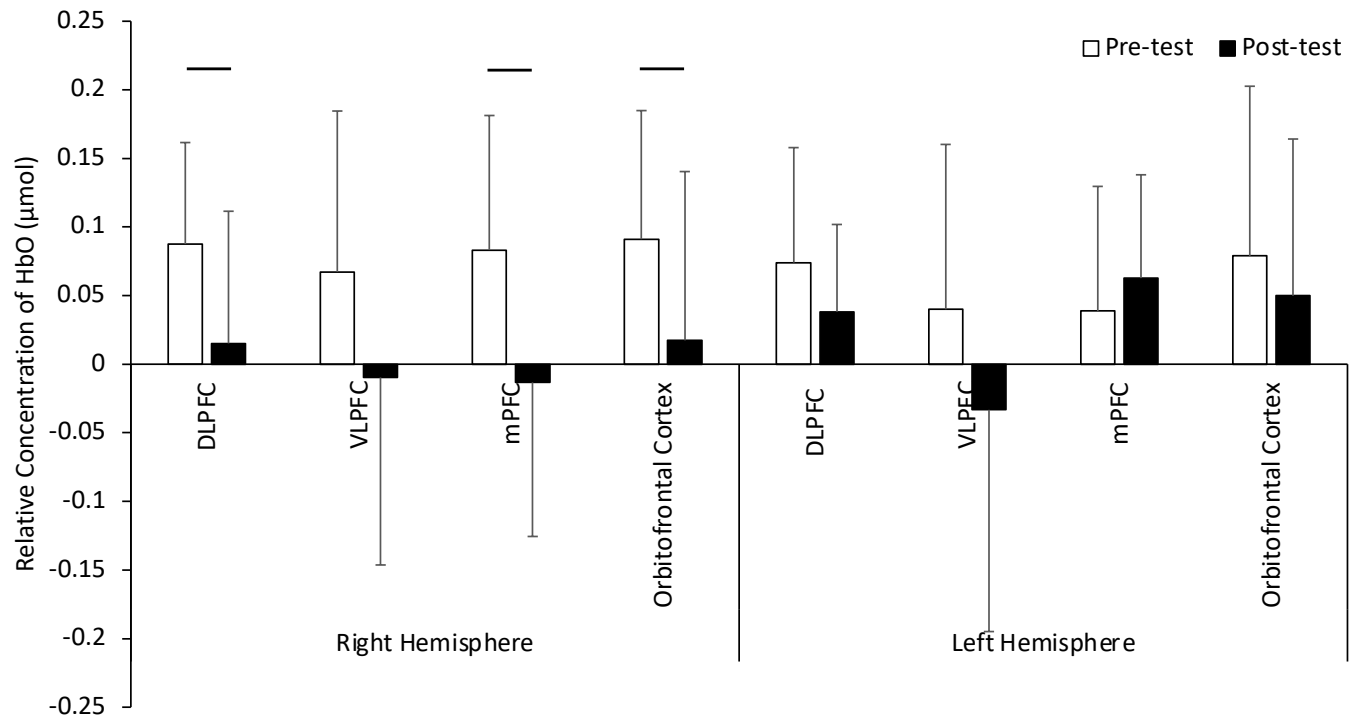


Figure 5.2. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for dual-task sequence-4 as a function of region and hemisphere. Horizontal line represents statistical significance ($p < 0.05$) (Refer to Chapter 5).

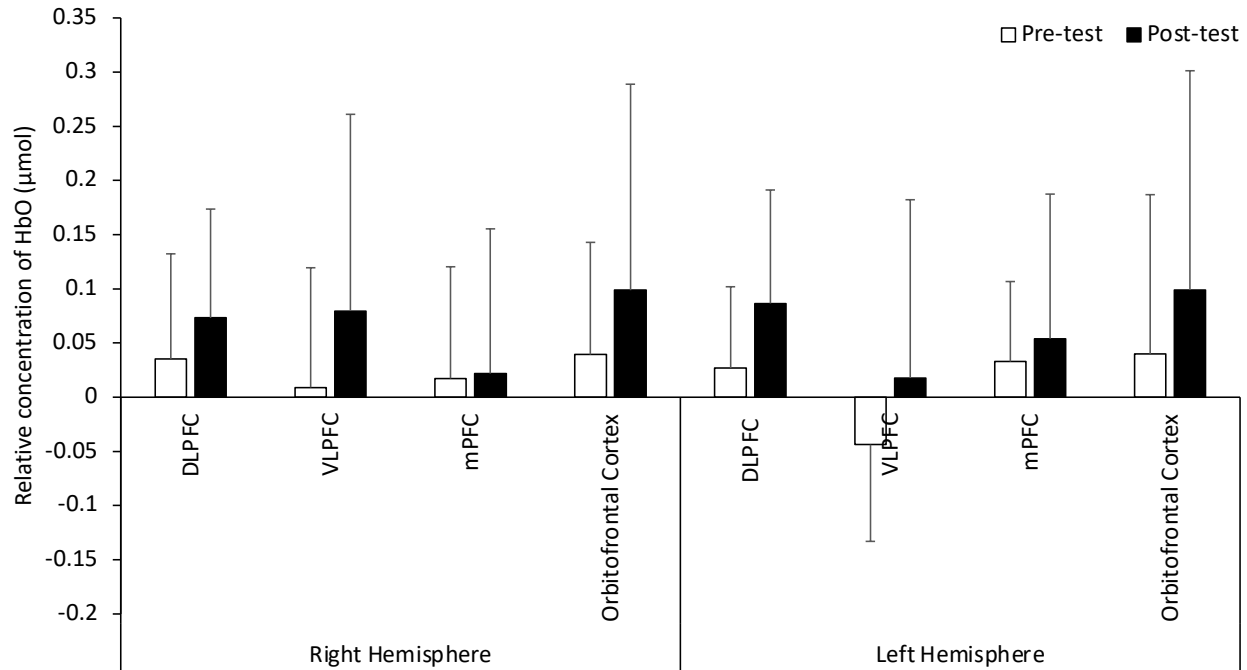


Figure 5.4. Change in relative concentration of HbO (mean $\pm$ SD) during pre-test and post-test for dual-task sequence-12 as a function of region and hemisphere (Refer to Chapter 5).

As previously stated, no significance was observed in the left hemisphere so we can only speculate about the increase in activation, but one possibility is that even with practice, the complexity of sequence-12 still required participants to attend to the preparation of their responses. As a result, increased activation of the left DLPFC was needed to meet the demand. It would then be expected that the dual-task sequence-4 exhibit reduced activation in post-test, considering the simplicity of the motor task. This was observed but as previously mentioned, failed to reach significance. Further examination is required to confirm the interpretations put forward in Chapters 2 and 5 with respect to the left DLPFC. The findings of this experiment (Chapter 5) were primarily limited to the right hemisphere and dual-task sequence-4. Of particular interest, was the decrease in activation in the right DLPFC and VLPFC from pre-test to post-test. The right VLPFC has previously been linked to dual-task interference (Herath,

Klingber, Young, Amunts, & Roland, 2001). Therefore, the change in VLPFC may represent a decrease in attentional demand, and a consequent decrease in dual-task interference as a result of practice. This is further supported by the behavioural data, which showed improved performance in the post-test for dual-task sequence-4 (Table 5.1., pg. 144). Another possible explanation for the practice-related decrease in DLPFC and VLPFC activity is a reduction in neural resources needed to select an appropriate response, considering the lateral PFC (DLPFC and VLPFC) has been implicated in response selection conflicts during dual-task performance (Erickson et al., 2005; Poldrack et al., 2005; Schumacher & D'Esposito, 2002). However, these are theories that will necessitate further investigation, especially with the right VLPFC yielding a marginal trend towards significance in Chapter 5.

Collectively, the findings of Chapters 2, 3, and 5 suggest the importance of caution when classifying sequential finger-tapping tasks as automatic. Presently, behavioural improvements appear to relate more to the degree of attentional demand than movement automaticity (Chapter 2, 3, and 5). For a motor task to be considered truly automatic, it would require months to years of practice (Fitts, 1964). Therefore, the decrease in the left DLPFC previously associated with automaticity (Wu et al., 2004) may actually reflect attentional investment in movement preparation (Chapter 3 and 5). Specifically, practice durations observed in the present dissertation did not make the task fully automatic, but rather reduced the attentional demand to a certain degree. Furthermore, we posit that the findings reflect the neural activation associated with reduced attentional demand and suggest that movement automaticity be considered as a continuum rather than a set construct.

6.2. Right hemisphere activation during motor sequence learning

The present dissertation revealed extensive right hemisphere activation following motor sequence learning. Specifically, Chapter 2 showed increased contributions from the right hemisphere including the DLPFC during S4 (Figure 2.1A, pg. 42) and S12 production, as opposed to no right hemisphere activation in Wu et al. (2004). While the differences cannot be readily explained, our results are consistent with several neuroimaging studies on motor sequence learning (Doyon et al., 1996; Doyon et al., 2002; Eliassen et al., 2001; Grafton et al., 1995; Hazeltine et al., 1997; Penhune & Doyon, 2002). As alluded to in the introduction, the aforementioned studies have commonly associated increased activation of the right PFC (e.g., DLPFC and VLPFC) to cognitive functions such as explicit learning, memory retrieval, sequential monitoring and spatial working memory. While all possible explanations for Chapter 2, assigning a particular function was difficult due to the limited significance between pre- and post-test, and the lack of coherence between HbO and HbR concentrations. However, the dominant theory was that the right hemisphere activation was reflective of explicit learning-related functions (Doyon et al., 1996; Eliassen et al., 2001; Hazeltine et al., 1997). The experiment in Chapter 3 enabled further examination of this hypothesis.

In Chapter 3, we found that for S1, the trained group exhibited greater activation in the right VLPFC during post-test compared to the pre-test. The opposite was observed in the control group with decreased activation during post-test and retention (Figure 3.3., pg. 82). This supports the suggestion that the right PFC is responsible for explicit motor sequence learning (Doyon et al., 1996; Grafton et al., 1995; Honda et al., 1998) and confirms our hypothesis. In addition, the VLPFC has been implicated in the active retrieval of explicit information from long-term memory (Petrides 1994; 1996; 2002). It would explain the increased activation during post-test, considering the trained group would have needed to retrieve the information acquired during

practice in order to perform the task. Chapter 3 also revealed reduced activation in the right orbitofrontal cortex during post-test and retention for S2 and S1, respectively, for the control group. The opposite was observed in the trained group, but failed to reach significance (Figure 3.4., pg. 83). We suspect that the reduced activation reflects the absence of motor sequence learning, as previous studies have implicated this region in the acquisition of sequential movements (Jackson, Lafleur, Malouin, Richards, & Doyon, 2003; Lafleur et al., 2002; Penhune & Doyon, 2002) and in selecting a response according to an intrinsic reward value. (Elliott, Dolan, & Frith, 2000).

Similar activations were expected in Chapter 5 but failed to occur. Interestingly, dual-task performance yielded decreased activation in the right hemisphere (DLPFC, mPFC, and orbitofrontal cortex) from the pre-test to the post-test for dual-task sequence-4 (Figure 5.2., pg. 145). Decreased activity in the right VLPFC was also observed but the significance was marginal ($p = 0.07$). The opposite pattern of activation was observed for dual-task sequence-12 but failed to reach significance. This resulted in explanations alternative to the ones presented in Chapters 2 and 3. While neuroimaging studies have attributed increased activation of the right hemisphere to explicit learning and active retrieval (Doyon et al., 1996; Grafton et al., 1995; Penhune & Doyon, 2002; Polskaia, St-Amant, Fraser, & Lajoie, 2020), we suggest that due to the simplicity of the S4 task, the processes mentioned above were not fully necessary to maintain dual-task performance in post-test (Remy et al., 2010). It would make sense then that dual-task sequence-12 exhibit opposing patterns, considering the task's increased complexity. Perhaps greater reliance on the PFC was needed to maintain the improved task performance following practice. However, as previously stated, the findings failed to reach significance for dual-task sequence-12.

Contrary to previous neuroimaging studies (Jackson et al., 2003; Lafleur et al., 2002; Penhune & Doyon, 2002), observing decreased activation of the orbitofrontal cortex during dual-task sequence-4 also required speculation as to its role. The reduced activation in post-test may reflect the neural demands associated with the visual letter-counting task. This is plausible considering our single-task imaging in Chapter 2 revealed that the visual letter-counting task overlapped with the finger-tapping task in the orbitofrontal cortex (Figure 6.3).

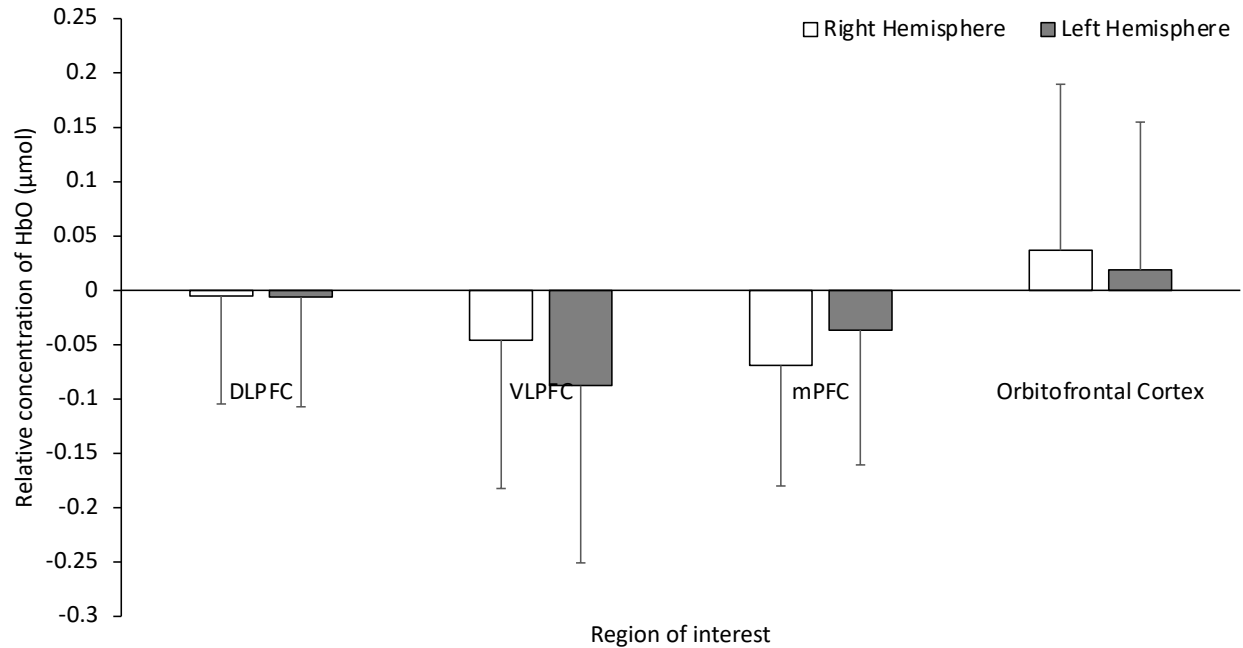


Figure 6.3. Change in relative concentration of HbO (mean $\pm$ SD) during the visual letter-counting task as a function of region.

Therefore, we suspect that in the post-test of dual-task sequence-4, attention was primarily placed on the visual letter-count task, and as a result, did not require increased contribution from a cortical region (e.g., orbitofrontal cortex) suggested to contribute to the acquisition of sequential motor skills (Jackson et al., 2003; Lafleur et al., 2002). For dual-task sequence-12, the increased activation in post-test, although statistically not significant, may imply that the neural demands associated with the motor task were still high following practice. Also, as previously mentioned, the medial orbitofrontal cortex has been suggested to be involved in selecting stimuli and/or response according to a reward value (Elliot, Dolan, & Frith, 2000). Therefore, the reduced activation in dual-task sequence-4 may also suggest that the visual letter-counting task provided minimal intrinsic reward. In terms of the mPFC, minimal activation was observed during post-test for dual-task sequence-4, which may suggest that participants were not engaging

in error processing (Menon, Adelman, Glover, & Reiss, 2001). However, at present, these remain working hypotheses that require further examination.

There are a few takeaways from the experiments in Chapters 2, 3, and 5. Explicit motor sequence learning exhibits right PFC activation, consistent with several previous neuroimaging studies (Doyon et al., 1996; Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998). Increased right VLPFC activity following practice may suggest participants are engaging in active retrieval of information from long-term memory (Petrides 1994; 1996; 2002). And finally, the neural representation of motor sequence learning during dual-task appears to be contingent on the complexity of the motor task. Overall, the experiments suggest that the right hemisphere is vital to the end of the fast/early learning phase (Chapter 2) and the slow/late learning phase of motor sequence learning (Chapter 3).

6.3. Issues regarding signal processing in fNIRS

A reoccurring issue in this dissertation was the high variability of the [HbO] measure. In most Chapters, the reported standard deviation was twice the mean. We hypothesized that motion artifacts and physiological contaminants were a major source of variability. In Chapter 2, we applied the principal component analysis (PCA), which identifies and removes the signal components that account for the greatest amount of spatial covariance (Wilcox et al., 2005; Zhang et al., 2005). The PCA was selected for its ability to significantly improve the mean-squared error and the contrast-to-noise ratio (CNR) of the recovered hemodynamic response function (HRF) (Cooper et al., 2012). However, the PCA did not appear to be very effective in reducing the overall standard deviation in Chapter 2 (refer to Figure 2.3A, pg. 46 for example). We suspect that some motion artifacts were localized to specific channels or temporally

correlated to the HRF, contributing to the poor performance (Brigadoi et al., 2014). In Chapter 3, we applied the wavelet filtering algorithm, which has been shown to be the most popular (Gemignani & Gervain, 2021) and most effective in reducing standard deviation, CNR, and area under the curve (Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al., 2015). As illustrated in Figures 3.3, 3.4, and 3.5 (pg. 82-85), certain conditions yielded high variability, compromising our ability to detect statistical differences if they did exist. The above mentioned performances could have also been impacted by the tuning parameters selected for the PCA (e.g., percentage of variance removed) and wavelet filter (e.g., interquartile range) functions. Ultimately, Chapters 2 and 3 highlighted the need for a comparison between available corrections to ensure accurate recovery and interpretations of the HRF when applied to motor sequence learning research. It is important to note that while logical steps were taken in selecting the PCA technique (refer to the limitations of section 2.5, pg. 55), we did not have a complete understanding of the difficulties (e.g., signal contamination) associated with pre-processing the fNIRS signal at the time. Specifically, the influence of the extracerebral layer on the quality of the fNIRS signal.

A comparison of corrections was explored in Chapter 4 of this dissertation. The objective was two-fold: first, compare the statistical outcomes of five motion correction techniques on data collected for Chapter 2 and second, determine which correction yields the best performance using metrics such as recovered HRF and within-subject standard deviation. Chapter 4 revealed that the choice of correction impacts the statistical results and, consequently, the conclusions drawn (Table 4.1-4.3., pg. 109-111). While all corrections produced a significant group x session x channel interaction (Table 4.1., pg. 109), only wavelet and spline + wavelet yielded a significant difference between pre-test and retention, enabling distinctions between learning and

performance. The analysis revealed that group comparisons would only be permitted with CBSI, as all other corrections were limited to the control group. Similarities were also observed but between a limited number of corrections (Table 4.2-4.3., pg. 110-111). We suspect the differences in results are related to the performance of each correction.

The findings associated with the second objective revealed no significant difference between corrections when compared across the recovered HRF (Figure 4.2., pg. 114). We suspect, that in addition to spike and baseline shift artifacts, our dataset contained motion artifacts that were challenging to detect due to their low frequency and amplitude. Spline interpolation, while effective at removing baseline shift (Hocke et al., 2018; Jahani, Setarehdan, Boas, & Yucel, 2018), requires prior motion artifact detection. The parameters set in the detection algorithm (`hmrMotionArtifactbyChannel`) may not have been effective at identifying this type of artifact (Brigadoi et al., 2014; Di Lorenzo et al., 2019). As a result, spline interpolation was not applied, and certain data points were processed with no correction. We drew a similar conclusion with the spline + wavelet, as it yielded HRFs comparable in shape to the wavelet filter (Figure 4.2, pg. 114). On its own, wavelet filtering was effective at removing spike artifacts but may have had difficulty distinguishing between the low-frequency motion artifacts, baseline shifts, and the HRF (Brigadoi et al., 2014). As a result, some wavelet coefficients could have been set to zero, leading to an underestimated HRF. In terms of PCA, we suspect the issues identified in Chapter 2 were likely the cause of poor performance in Chapter 4. With CBSI, we suggest that data points unrelated to motion were removed simply do to their positive correlation, contributing to the attenuated response. Studies have shown cerebral blood flow (CBF) to precipitate a simultaneous increase in HbO and HbR following cortical activation (Fujiwara et al., 2004; Murata, Sakatani, Katayama, Fukaya, 2002). In addition, the corrections

yielded limited significance when compared using within-subject standard deviation (Table 4.4 – 4.5., pg. 117-118). CBSI did produce the lowest standard deviation but should be interpreted with caution, as we suspect the low concentration values were a result of over filtering. As expected, no correction method (i.e., bandpass) produced the highest standard deviation compared to wavelet, spline + wavelet, and CBSI. The findings also revealed decreased standard deviation with wavelet and spline + wavelet when compared to spline alone (Table 4.4-4.5, pg.117-118) and in the left DLPFC channel when compared to bandpass and spline (Table 4.6, pg.119). However, we cannot readily explain why this significance was limited to certain conditions. Further research is required to determine if wavelet filtering has the potential to be a standardized pre-processing method.

Therefore, with limited significance, only a few recommendations can be put forward. First, the findings suggest that corrections that require prior motion detection (e.g., spline) or independence between extracerebral and cerebral layers (e.g., PCA) may not be suitable for motor sequence learning experiments. Alternatively, parameters could be selected on a participant-by-participant basis to improve artifact detection, but this approach should be carefully considered as it can introduce subject bias. Second, the experiment also demonstrated the importance of selecting appropriate tuning parameters such as cut-off frequencies (e.g., low-pass and high-pass) for bandpass, interquartile range for wavelet, and percentage of total variance for PCA. With variability among studies (Behrendt et al., 2018; Brigadoi et al., 2014; Cooper et al., 2012; Di Lorenzo et al., 2019; Hu et al., 2015), and poor reporting (Pinti, Scholkmann, Hamilton, Burgess, & Tachtsidis, 2019), it is important to determine which tuning parameter is most applicable. For motor sequence learning studies, recommendations provided by comparison studies should only be used as a guide. Once a correction is chosen (based on the

artifacts present in the signal), all parameters should be evaluated to determine which parameter provides the best compromise between artifact removal and HRF recovery. Therefore, for Chapter 5, we decided to keep the wavelet parameter due to its limited success in Chapter 4 but refine the bandpass cut-off frequencies based on the recommendation put forward by Pinti et al. (2019). The low-pass was lowered from 0.5 Hz to 0.09 Hz, which still preserved the simulation frequency but removed additional physiological contaminants such as respiration (~ 0.3 Hz) and Mayer waves (~ 0.09 Hz) from the signal. The result was a smoother signal, but it did not appear to significantly minimize the standard deviation.

6.4. Limitations and future directions

There are several limitations of note with the present dissertation. First, was the structure of the familiarization period in Chapter 3. Preceding each acquisition run (Figure 3.2B, pg. 86) with a familiarization period may have skewed the baseline performance by enabling participants to retain and rehearse the sequential knowledge in short-term memory. This may explain why the pre-test and the post-test error scores were relatively similar for the trained group and why it was challenging to distinguish learning from performance. Second, was the limited number of active blocks and comparison metrics in Chapter 4. It is possible that having too few blocks (e.g., 3) contributed to the noisy hemodynamic response (Brigadoi et al., 2014). With limited blocks, block averaging could only do so much, in terms of improving the signal-to-noise ratio and reliability of the fNIRS signal. Additionally, the fact that different techniques produced different statistical outcomes but yielded no differences in the recovered HRF may suggest that certain aspects of the HRF were different between techniques. Including metrics that compare the size or shape of the HRF, such as peak amplitude (Gemignani & Gervain, 2021), CNR (Cooper et al.,

2012), or area under the curve (Brigadoi et al., 2014), may help to better elucidate performance differences. Third, was signal contamination from evoked and non-evoked systemic sources at the cerebral and extracerebral layer (Scholkmann et al., 2014). This includes blood pressure, cerebral blood flow, skin blood, heart rate, respiration, Mayer waves, and low frequency oscillations. While the latter four contributors are primarily not task-related, their characteristics (e.g., amplitude, frequency, etc.) can be influenced by the task (Franceschini, Fantini, Toronov, Filiaci, & Gratton, 2000; Scholkmann et al., 2014). Finger-tapping, in particular, has been found to cause heart rate, arterial pulse, and respiration to synchronize with the stimulation frequency (Franceschini et al., 2000). While bandpass filtering and block averaging can be used to remove some of the non-evoked systemic components (heart rate, respiration, Mayer waves, and low frequency oscillations), they cannot eliminate the extracerebral-related noise. Future experiments should implement short-optode separation channels (1 – 2 cm) that can be used to regress out extracerebral effects from the overall fNIRS signal (Tachtsidis & Scholkmann, 2016). Fourth, differences in scalp-brain distance between participants may have contributed to the variability in the observed signal (Cui et al., 2011). Larger scalp-brain distances have been shown to reduce the CNR and correlation with the BOLD signal. Finally, sex differences may have contributed to the variability, as previous research has shown males to exhibit higher overall [HbO] than females (Cinciute, Daktariunas, & Ruksenas, 2018).

Therefore, based on our collective findings, we suggest that fNIRS can be capable of detecting differences in the PFC associated with motor sequence learning, if careful steps are taken. We recommend future studies to implement multi-modal imaging (fNIRS-fMRI) with the inclusion of short-optode channels. This will enable comparison between techniques within the same protocol and the regression of extracerebral noise. In addition, all parameters for a

correction should be tested, considering motor sequence learning contains motion artifacts of varying shape and frequency (e.g., high-frequency spikes, baseline shifts, slow drifts, and low-frequency artifacts).

6.5. Concluding remarks

The results from this dissertation provide insight into the contributions of the PFC to motor sequence learning and movement automaticity. The findings suggest that 1) explicit motor sequence learning and retrieval of information from long-term memory requires contribution from predominately the right hemisphere; 2) activation of the left DLPFC may reflect attentional investment in movement preparation rather than movement automaticity, and 3) the neural representations of dual-task processing are contingent on the complexity of the motor sequence task. In terms of fNIRS, the dissertation and Chapter 4, in particular, highlights the signal processing issues that still need to be resolved to ensure HRF data is accurately interpreted and the processing steps that need to be taken when applying fNIRS to motor sequence learning studies.

6.6. References

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7. Appendices

7.1. Ethics approval for Chapters 2 and 5

File Number: H04-17-15

Date (mm/dd/yyyy): 05/03/2017



Université d'Ottawa
Bureau d'éthique et d'intégrité de la recherche

University of Ottawa
Office of Research Ethics and Integrity

Ethics Approval Notice **Health Sciences and Science REB**

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

<u>First Name</u>	<u>Last Name</u>	<u>Affiliation</u>	<u>Role</u>
Yves	Lajoie	Health Sciences / Human Kinetics	Principal Investigator
Sarah	Fraser	Health Sciences / Others	Co-investigator
Nadia	Polskaia	Health Sciences / Human Kinetics	Co-investigator

File Number: H04-17-15

Type of Project: Professor

Title: How self-initiated memorized movements become automatic: A functional near-infrared spectroscopy (fNIRS) validation and replication study

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
05/03/2017	05/02/2018	Approval

Special Conditions / Comments:
N/A

7.2. Ethics approval for Chapters 3 and 4

Université d'Ottawa

Bureau d'éthique et d'intégrité de la recherche

University of Ottawa

Office of Research Ethics and Integrity

CERTIFICAT D'APPROBATION ÉTHIQUE | CERTIFICATE OF ETHICS APPROVAL

Numéro du dossier / Ethics File Number	H-05-19-4019
Titre du projet / Project Title	Cortical activation of motor sequence learning examined with functional near-infrared spectroscopy
Type de projet / Project Type	Thèse de doctorat / Doctoral thesis
Statut du projet / Project Status	Renouvelé / Renewed
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Équipe de recherche / Research Team

Chercheur / Researcher	Affiliation	Role
Nadia POLSKAIA	École des sciences de l'activité physique / School of Human Kinetics	Chercheur Principal / Principal Investigator
Yves LAJOIE	École des sciences de l'activité physique / School of Human Kinetics	Superviseur / Supervisor

Conditions spéciales ou commentaires / Special conditions or comments

550, rue Cumberland, pièce 154 550 Cumberland Street, Room 154
Ottawa (Ontario) K1N 6N5 Canada Ottawa, Ontario K1N 6N5 Canada

613-562-5387 • 613-562-5338 • ethique@uOttawa.ca / ethics@uOttawa.ca
www.recherche.uottawa.ca/deontologie | www.recherche.uottawa.ca/ethics