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An exploration of dual task benefits to motor learning and  
effects on prefrontal cortex haemodynamics

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This Thesis has been completed as a requirement for a  
postgraduate research degree at the University of Winchester

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## Abstract

The use of a dual task protocol has the potential to enhance performance and learning of a novel motor skill, although the neurological mechanisms behind this are not currently understood. The aim of this research was to establish whether a simple dual task protocol enhanced performance and learning of a novel continuous motor skill, and whether the effects of the dual task on neurological responses could be inferred from the haemodynamic response within the prefrontal cortex. Five studies were conducted to investigate the effects of a simple dual task on novel skill performance, to assess the validity and reliability of a single position near infrared spectroscopy device to measure the haemodynamic response in the prefrontal cortex, to examine the effects of a simple dual task protocol on novel skill learning and to determine whether the mechanisms behind the dual task responses could be inferred from the haemodynamic response in the prefrontal cortex. The findings of this research indicated that dual tasks do not aid novel skill performance, however training in a simple dual task condition is beneficial to skill learning. Furthermore, improved performance after training in a dual task condition was maintained for four weeks following the end of training. The findings also indicated that whilst there is some evidence to support the validity of single position near infrared spectroscopy (NIRS) for determining haemodynamic changes in the prefrontal cortex the validity of this measure could not be fully established. Furthermore, although within day reliability of the single position NIRS was acceptable, between day reliability was poor. The neural mechanisms behind the responses to the dual task protocols were not established, although there was initial evidence to suggest that training in a more challenging dual task condition induces a greater level of mental effort and is less beneficial to skill learning. In conclusion, a simple dual task protocol can enhance motor skill learning and whilst single position near infrared spectroscopy may have some benefits for assessing haemodynamic responses to a cognitive stimulus, the validity and reliability of this device have not been fully established.

Keywords: Skill learning, NIRS, cerebral blood flow, oxygenation, skill performance

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## Glossary of common abbreviations

[Hb <sub>diff</sub> ]	haemoglobin difference
[HHb]	deoxyhaemoglobin
[Mb]	myoglobin
[O <sub>2</sub> Hb]	oxyhaemoglobin
[tHb]	total haemoglobin
[TSI]	tissue saturation index
BOLD fMRI	blood oxygen level dependent functional magnetic resonance imaging
CBF	cerebral blood flow
CSF	cerebrospinal fluid
cw-NIRS	continuous wave near infrared spectroscopy
DLPFC	dorsolateral prefrontal cortex
DTI	dual task interference
EEG	electroencephalogram
fMRI	functional magnetic resonance imaging
fNIRS	functional near infrared spectroscopy
GET	gas exchange threshold
GXT	graded exercise test
MBLL	modified Beer-Lambert law
NIR	near infrared light
NIRS	near infrared spectroscopy
PET	positron emission tomography
PFC	prefrontal cortex
PRP	Psychological Refractory Period
rCBF	regional cerebral blood flow
RPM	revolutions per minute
SOA	stimulus onset asynchrony

## Forward

A thesis is always more than a collection of words and data, it is the summation of a journey of research and exploration. At times this journey is straightforward, and this is reflected in the finished thesis where the rationale behind every decision is clear and easy to follow. At other times the journey from the start of the thesis to the endpoint is more convoluted and at such times the rationale for the decisions made is less clear. That has been the case for this thesis where the actual direction taken during the process of this research was somewhat removed from the initial intended direction. Consequently, the rationale for some of the decisions made, particularly in the early stages, has impacted on the later chapters in a way that may not be initially clear to the reader. Therefore, this forward will explain the journey taken throughout this body of research in order to clarify the process undertaken and the decisions made.

The initial aim of this body of work was to culminate with an intervention designed to aid the recovery of stroke survivors. This was based on the conclusion of Hemond, Brown and Robertson (2010) and Goh, Sullivan, Gordon, Wulf and Winstein (2012) that dual tasks can enhance performance and learning of a motor skill by engaging similar neural networks. This conclusion inspired the hypothesis that stroke recovery may be aided by the engagement of similar neural networks which could help to compensate for the neurological regions damaged during a stroke. Since the use of a dual task had been shown to aid skill learning in healthy populations (Roche et al., 2007; Goh et al., 2012) it was hypothesised that the availability of more neurological resources would have further benefit to stroke survivors by aiding the relearning of motor skills. This hypothesis was supported by recent studies which have shown benefits of using dual task training in the rehabilitation of lower limb impairment following stroke (An et al, 2014; Choi, Lee & Lee, 2015). Whilst lower limb impairment is a serious issue following stroke, the most common impairment is to the upper limb (Sabini, Dijkers & Raghavan, 2013; Sugg, Müller, Winstein, Hathorn, D. & Dempsey, 2015) and therefore this thesis aimed to develop an intervention that would aid upper limb rehabilitation.

The particular aim was to work with stroke survivors who had been discharged from rehabilitative care due to reaching a plateau in recovery and in particular to develop an intervention that could be completed within the patients' own home as commuting to a rehabilitative facility can be problematic for stroke survivors and it is also difficult to replicate the home environment in a rehabilitative setting (Hillier & Inglis-Jasiem, 2010).

One component of the aim to develop a home-based rehabilitation intervention was to utilise a primary task that was easily accessible to stroke survivors and which would be fairly low cost to provide. This led to the decision to use an active video game which specifically targeted upper limb movements. The Xbox Kinect™ is a system which allows users to operate the game without the use of a controller and the reliability of the Kinect system has led to this active gaming system being utilised in rehabilitation settings (Park, Lee, Lee & Lee, 2017). The specific game chosen for use in this thesis was a bowling game which had the potential to promote upper limb mobility in stroke survivors.

Whilst the bowling game chosen may have provided a useful tool for rehabilitation, the goal of developing a rehabilitation programme for stroke survivors was not realised due to the necessity to validate near infrared spectroscopy equipment which formed the second part of this planned programme of research. The fact that this thesis ultimately culminated in the examination of the effects of dual task training in healthy populations meant that the bowling game was only utilised as a primary task in healthy populations. As an active video game is primarily aimed to provide entertainment not to be utilised in a carefully controlled scientific experiment the use of this game may have led to some limitations in the results obtained. If the goal of this programme of research had been solely to provide a proof of concept in healthy populations an alternative primary task would have been utilised and therefore more robust results may have been obtained.

The failure to reach the goal of developing a rehabilitation programme was predominantly influenced by the chapters which examined the validity and reliability of a single position near infrared spectroscopy (NIRS) device. The decision to utilise this device in this body of research was based on two separate intentions. Firstly, the intention was to attempt to understand the neurological mechanisms underpinning the benefits observed in previous studies in relation to dual task training. The work of Goh et al. (2012) and Hemond, Brown and Robertson (2010) hypothesised that the dual task benefits to motor learning occur due to the engagement of similar neurological networks, however, no evidence had been produced to support this hypothesis. The decision to examine the NIRS rather than utilise a more established neuroimaging tool was primarily rooted in the second intention, which was to examine any changes in responses in stroke survivors for whom changes in blood flow may be an important indicator of recovery (Prakash & Carmichael, 2015). Although there are a number of different NIRS devices which have been developed, the use of this tool within this programme of research was limited to the use of a single position NIRS which was the device available within the university. This device, however,

had not been validated for use in cognitive research which necessitated an examination of the validity and reliability of the device. This necessity delayed the use of this device for examination of dual task effects and the time taken to examine the use of this device meant that the programme of research was unable to progress to examine the effects of dual task training in stroke survivors.

One additional aspect of the chapters examining the use of the NIRS device which may not be immediately apparent to the reader is the inclusion of exercise testing. This variable was included for two reasons. First, the active video game used meant that the participant was moving substantially whilst completing the task. Although NIRS is fairly robust to movement artefacts (Hoshi, 2011) movement can affect blood flow changes in the brain (Robertson & Marino, 2016) and therefore the validity and reliability of the NIRS signal may have been impacted by the movement during the task. The second reason for including this aspect in the study design stems from the original intention to work with stroke survivors. If the protocol was to be used during a rehabilitation setting there would be a necessity to record accurate data during movement/physical activity and consequently it was necessary to ensure the data obtained from the NIRS device during activity was both valid and reliable.

Whilst the progress of this thesis did not culminate in the way that was intended at the start of this programme of research the design of studies was influenced throughout by this intended goal. Therefore, although on first examination the rationale for decisions made in this thesis may not be clear I hope that this forward will serve to provide context for the reader. Furthermore, this forward should serve to provide a starting point from which to interpret the results obtained.

## Chapter 1: Introduction

Interest in the optimum ways to learn and develop new skills is longstanding, and many methods have been examined by researchers attempting to accelerate or enhance the quality of the acquisition of novel skills. One area of research that has helped to elucidate the mechanisms involved in learning a skill is the understanding of the effects of dual tasks which are often used as a measure of the automaticity of a learnt skill (Logan, 1985; Poldrack et al., 2005; Taatgen, 2005). Life is characterised by situations where the requirement to perform more than one task at a time occurs, and most people are able to do this without any noticeable effects (Donohue, James, Eslick & Miroff, 2012). In some situations, doing two things at a time are virtually effortless (e.g., walking and talking) and in others the successful combination of tasks is extremely difficult or even dangerous (e.g., driving and texting) (Salvucci & Taatgen, 2008). Developing and extending understanding of the ability to perform two tasks concurrently provides useful information on how the human brain is able to focus attention and process multiple cognitive demands (Karatekin, Couperus & Marcu, 2004). Furthermore, the use of a dual task paradigm has contributed to an understanding of cognitive aging (Bherer et al., 2005; Verhaeghen & Cerella, 2002), balance and control in elderly populations (Hiyamizu, Morioka, Shomoto & Shimada, 2012; Silsupadol et al., 2009), diagnosis of dementia (Montero-Odasso et al., 2017; Naidu, Vasudev, Burhan, Ionson & Montero-Odasso, 2019) and rehabilitation of neurological conditions such as stroke, traumatic brain injury (TBI) and Parkinson's disease (Howell, Osternig & Chou, 2013; O'Shea, Morris & Ianchek, 2002; Yang, Chen, Li, Cheng & Wang, - 2007).

### 1.1 Dual task interference (DTI)

The term dual task is used to refer to the consecutive completion of two tasks which often have disparate cognitive processing requirements or sensory inputs (Adcock, Constable, Gore & Goldman-Rakic, 2000). The presence of a secondary task generally creates an effect known as dual task interference (DTI) which causes impairment to the performance of one or both tasks (Chen et al., 2013; Goh, Ewing, Marchuk, Newton & Nyangani, 2019; Karatekin, Couperus & Marcus, 2004; Leone, Feys, Moumdjian, D'Amico, Zappia & Patti, 2017). This secondary task usually involves either a working memory component (e.g., counting backwards) or the requirement for a response to a presented stimulus (e.g.,

pressing a button in response to an audio or visual stimulus) (Brown, 1997; Töllner, Strobach, Schubert, & Mueller, 2012).

## **1.2 The effect of dual tasks on skill performance and learning**

Within the context of skill performance and acquisition dual tasks are generally considered only as a measure of the automaticity of the learnt skill, or as a way to distinguish between novice and expert performers (Beilock, Carr, MacMahon & Starkes, 2002; Beilock, Wieranga and Carr, 2002). When a skill is novel the learner is said to benefit from a skill focused environment (Schaefer, 2014) and consequently the presence of a secondary task is considered to be detrimental to skill performance (Chen et al., 2013; Huestegge & Koch, 2010; Pashler, 1994a). Therefore, despite extensive research into the importance of an external focus of attention in the performance and learning of new skills (see Wulf, 2013 for a review), the use of dual tasks to facilitate this external focus has not been extensively examined. There are, however, a limited number of studies which have shown the benefits of dual tasks in both novel skill performance (Hemond, Brown & Robertson, 2010) and skill learning (Chiou & Chang, 2016; Goh et al., 2012; Roche et al., 2007). It has been indicated that these benefits stem from the activation of similar neurological regions, facilitating a greater availability of neuronal resources for completion of the primary task (Goh et al., 2012; Hemond et al., 2010). These studies have focused on short duration, discrete motor or visual learning tasks and therefore the results may not be applicable to longer duration continuous motor skills which are more complex in nature than discrete tasks (Gopher, Brickner & Navon, 1982; Maynard & Hakel, 1987; Oberauer & Kliegel, 2004; Rice et al., 2012; Ruthruff, Pashler & Johnson, 2001). Furthermore, although it has been hypothesised that the activation of similar neural networks plays a role in dual task enhanced performance and learning (Goh et al., 2012; Hemond, Brown & Robertson, 2010), no objective measures of neural activation have been examined in relation to dual task benefits.

## **1.3 Neurological mechanisms underpinning dual task interference**

In recent years there has been an increasing interest in the neurological mechanisms underpinning the effect of dual tasks, with systematic reviews examining literature from both human (Leone et al., 2017) and animal studies (Watanabe & Funahashi, 2018). No consensus yet exists, either in relation to the neural mechanisms involved, or in the brain regions responsible for DTI, although there is an indication of consistent involvement of the

prefrontal cortex (PFC) (Leone et al., 2017). It is unclear, however, whether this involvement is due to a specific role of the PFC in mediating dual task interference or a general uprating of activation in response to increased cognitive demands (Van Impe, Coxon, Goble, Wenderoth & Swinnon, 2011).

#### **1.4 Neuroimaging and near infrared spectroscopy (NIRS)**

There are several neuroimaging techniques used to examine neurological responses to a cognitive stimulus, the most common of which are blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI), electroencephalography (EEG) and near infrared spectroscopy (NIRS). BOLD fMRI uses the paramagnetic properties of deoxyhaemoglobin [HHb] to examine changes in haemoglobin status within the blood which is then used to determine brain activity (D'Esposito, Deouell & Gazzaley, 2003; Kim & Bannettini, 2012; Logothetis & Pfeuffer, 2004). BOLD fMRI scanners have the advantage of high spatial resolution and can produce whole brain imaging, however, they are high cost, require subject immobility and need extensive training to operate (Scarappicchia, Brown, Mayo & Gawryluk, 2017). Rather than monitoring blood flow changes in the brain in response to neural activation, the EEG relies on electrical signals generated by nerve pulses (Sutter, Caplan & Schomer, 2017). Although lacking the spatial resolution of the BOLD fMRI scanners, EEG has high temporal resolution and is accurate in determining local neural activity (Arefian, Seddighi, Seddighi & Zali, 2012; Cook, O'Hara, Uijdehaageac, Mandelkern & Leuchter, 1998; Cuffin et al., 1991; Walczak, Radtke & Lewis, 1992). Whilst allowing for more movement than the BOLD fMRI scanners, the EEG is highly subjective to movement artefacts (Butti et al., 2006; Canning & Scheutz, 2013; Teplan, 2002) and therefore lacks ecological validity.

Near infrared spectroscopy (NIRS), however, is relatively robust to movement artefacts and has high levels of ecological validity (Ferrari et al., 2014; Hoshi, 2011; Kakimoto et al., 2009). NIRS uses light in the infra-red spectrum to examine haemodynamic responses within the region of interest (Boas, Elwell, Ferrari & Taga, 2014) and can be used to assess the neurological responses to a stimulus. In order to examine changes in neurological activation NIRS relies on the tight coupling between neural activity and haemodynamic response (Yanagisawa et al., 2010). Specifically, increases in oxygen delivery to an area of the brain can be used to infer an increase in neural activity within that region (Strait & Scheutz, 2014). There are many commercially available NIRS devices, some of which are able to simultaneously measure multiple regions of the brain. However, one of

the most accessible and affordable devices uses the technology to measure responses within one specific region at a time. These devices are often used in sports science research for the assessment of changes in muscle oxygenation (Fryer et al., 2016; Jones & Cooper, 2014; Júnior et al., 2015) and therefore the ease of access to these devices has led to their use in examination of neurological responses both to physiological manipulations and cognitive stimuli (Porcelli, Marzorati, Lanfranconi, Vago, Pisot & Grassi, 2010; Rupp et al., 2013; Smith & Billaut, 2010; Subudhi, Dimmen & Roach, 2007). There have been several investigations into the validity and reliability of the multiple region NIRS devices (e.g., Alderliesten et al., 2014; Claassen, Colier & Jansen, 2006; Kono et al., 2007; Mehagnoul-Schipper et al., 2002), however, there is currently an absence of research investigating the validity and reliability of the single position devices, such as the Artinis Portalite NIRS device, for cognitive research. In order for a measurement tool to be useful in the acquisition of data it is important that it first be demonstrated to a) measure the concept or response that it purports to measure and b) produce results which are both consistent and repeatable (Gratton & Jones, 2009) and therefore establishing the validity and reliability of the Artinis Portalite NIRS device is highly important. Furthermore, as haemodynamic responses may differ in response to exercise (Ekkekakis, 2009) it is important to establish whether the Artinis Portalite NIRS device is a valid and reliable tool for assessing haemodynamic responses to a cognitive stimulus during exercise particularly since the primary task used in chapters 3 and 7 includes an active video game.

### **1.5 Thesis Aims**

This thesis aimed to address the following gaps identified in the literature by addressing a number of specific research questions:

1. *Aim:*

- To examine whether a dual task which is expected to activate similar neurological processes as the primary task could be used to facilitate novel skill performance.

*Research question:*

- Can a secondary audio response task presented during a continuous motor skill improve novel skill performance?

2. *Aim*

- To investigate the psychophysiological mechanisms underpinning dual task effects

Research question:

- Is pupillometry a suitable technique for determining the psychophysiological responses to dual task interventions?

3. *Aim:*

- To examine whether a dual task which is expected to activate similar neurological processes as the primary task could be used to facilitate novel skill learning.

*Research question:*

- Will training in dual task conditions improve novel skill learning compared to training in a single task condition and is learning dependent on dual task type?

4. *Aim:*

- To examine whether retention of a learnt skill is facilitated by the presence of a dual task at retention.

*Research question:*

- Does having a dual task present during retention aid performance of a learnt skill?

5. *Aim:*

- To explore whether training in dual task conditions alters the haemodynamic response to neurological activation and whether this activation differs dependent on dual task type.

*Research questions:*

- Do different dual task protocols effect the haemodynamic response during novel skill performance?
- Is there an effect of training group on the haemodynamic response during immediate and delayed skill retention tests?

6. *Aim:*

- To examine the optimum way of processing data obtained by the Artinis Portalite NIRS device at rest and during exercise

*Research questions:*

- Does absolute data provide a useful determinant of haemodynamic responses to a cognitive stimulus at rest and during exercise?
- Which method of relative data processing provides the most accurate determinant of the haemodynamic response to a cognitive stimulus at rest and during exercise?

7. *Aim:*

- To investigate the validity of the Artinis Portalite NIRS device in determining - haemodynamic responses to neural activity.

*Research question:*

- Do the responses to neural activation recorded by the Artinis Portalite NIRS at rest device correlate with those recorded by an EEG?
- Do the responses to neural activation recorded by the Artinis Portalite NIRS during exercise correlate with behavioural measures in the same manner as those recorded at rest?

8. *Aim:*

- To examine the between and within day reliability of the Artinis Portalite NIRS device at rest and during exercise

*Research questions:*

- Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements at rest on the same day?
- Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements at rest on different days?
- Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements during exercise on the same day?
- Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements during exercise on different days?

9. *Aim:*

- To investigate the optimum positioning of a single position NIRS device to determine neural activation in the prefrontal cortex

*Research question:*

- Which region of the prefrontal cortex is activated in response to the Stroop colour word task?

## **1.6 Thesis structure**

This thesis will begin with a review of the relevant literature regarding dual tasks and novel skill performance and learning as well as the literature surrounding NIRS which will be presented in chapter two. This thesis also includes five experimental chapters. The first empirical chapter (chapter 3) addresses aims one and two by examining the influence of two different dual tasks on performance of a novel skill (an active video game) and

assessing the use of pupillometry for determining the psychophysiological responses to a dual task protocol. This chapter also examines whether changes in pupillary responses can be used to identify differences in dual task demands. The second empirical chapter (chapter 4) addresses aim 4 by investigating the optimum way to process NIRS data using a cognitive task applied at rest and during exercise and examining three different methods of processing the NIRS data obtained and contributes to answering the research questions associated with aim nine. The third experimental chapter (chapter 5) addresses aims seven and nine by examining the haemodynamic responses to a cognitive stimulus at rest and during exercise in four different brain regions and comparing the results obtained at rest to neurological responses to the same cognitive stimuli measured using an EEG at rest.

The research questions associated with aims eight and nine are addressed in the fourth experimental chapter (chapter 6) which examines the reproducibility and reliability of NIRS measurements in response to a cognitive stimulus at rest and during exercise and examines the haemodynamic responses on an individual trial basis. A repeated measures design is used to determine within day and between day reliability. The final experimental chapter (chapter 7) addresses the research questions associated with aims one, three, four and five. The NIRS device is used to assess haemodynamic responses to the performance of four different dual tasks at baseline and following a period of skill training. Performance tests applied at baseline and at three separate intervals post training are used to determine skill learning. Training is conducted in three different conditions and performance improvements are compared between conditions. The thesis concludes with a general discussion and summary of the results obtained, with a discussion of the limitations of this body of work and the identification of directions for future research.

## Chapter 2: Review of the Literature

### 2.1. Characteristics of skill learning

The requirement to efficiently acquire new skills occurs at all stages of life and is of critical importance to the ability to grow and develop (Kantak & Winstein, 2012). When a skill is learnt, progression from a slow deliberate form of processing to quicker, more automated processing occurs (Taatgen, 2005). In the early stages of skill learning a large amount of cognitive control is required in order to coordinate all the components required for task execution (Taatgen, Juvina, Schipper, Borst & Martens, 2009). It is thought that the cognitive representation of the skill becomes increasingly efficient when the skill is repeatedly performed (Taatgen, 2005), and the high attentional demands present in the early stages of learning dissipate as the skill is extensively practiced (Brown & Bennet, 2002; Evans & Graham, 1980). The development of a skill is thought to occur essentially in two phases, an initial phase in which performance improves rapidly over initial training sessions, and a slower phase of improvement that occurs over an extended period of time (Karni et al., 1998; Kleim et al., 1996; Kleim et al., 1998; Nudo et al., 1996b).

The most prominent model of skill learning is the three stage theory of skill acquisition (Fitts & Posner, 1967), which consists of a cognitive stage, an associative stage and an autonomous stage. The early stage of this process (cognitive stage) is said to involve the development of cognitive processes for skill identification, response selection and skill execution (Kantak & Winstein, 2012). This stage is thought to be primarily governed by implicit processes which occur without awareness of the action (Knowlton, Mangels & Squire, 1996). This stage of learning is characterised by frequent errors, and implicit mechanisms within the brain use knowledge of the results of previous attempts to allow gradual adjustment of the skill in order to improve performance (Klinberg, 2010; Magill & Anderson, 2014). The second stage of the model, the associative stage, is also referred to as the refining stage, where the priority is consistency (Magill & Anderson, 2014). The final stage of the model, the autonomous stage, is where the skill has become automatic (Magill & Anderson, 2014). This is the stage where the performer is likely to be able to manage the performance under a higher cognitive load (Schaefer, 2014) and it is characterised by accuracy, reliability and consistency of skill execution (Wulf, 2013).

One of the key components of learning a new skill is practice, not just the amount but also the structure and nature of practice sessions (Kantak & Winstein, 2012). In contrast

to what might be expected, some practice types that enhance performance immediately do not lead to long term improvements, whereas practice types which impair initial performance can enhance long term learning (Schmidt & Bjork, 1992; Lee & Wishart, 2005).

### **2.1.1 Assessment of learning**

Learning cannot be measured but rather it is inferred from changes in performance and a careful distinction between these two concepts is essential for studies assessing skill acquisition (Cahill, McGaugh & Weinberger, 2001; Katak & Winstein, 2012). When assessing learning it is vital to control for factors such as attention, motor function and arousal level (Cahill, McGaugh & Weinberger, 2001). The distinction between learning and performance is most apparent when practice conditions are challenging and therefore initial performance is impaired, but performance in a delayed retention test is improved (Katak & Winstein, 2012). Learning is said to have occurred when there is a relatively permanent change in performance of the skill which is sustained over time, and when assessing learning it is important to distinguish between transient performance gains and sustained, consistent performance (Cahill, McGaugh & Weinberger, 2001; Salmoni, Schmidt & Walter, 1984; Schmidt & Bjork, 1992; Schmidt & Lee, 2004). In order to ascertain this, it is crucial to leave a suitable interval between the end of practice and any tests of performance to ensure that any transient performance gains have dissipated (Katak & Winstein, 2012).

One common way of assessing learning is by using a retention test which is when performance is examined in the same conditions that were present during practice (Katak & Winstein, 2012; Saemi, Porter, Ghotbi-Varzaneh, Zarghami & Maleki, 2012). This is a measure of how well the skill is retained over the interval between practice and retention (Katak & Winstein, 2012; Wulf, Shea & Lewthwaite, 2010). An alternative way of assessing learning is by using a transfer test, which may involve performing a new but similar skill or a new variation of the practiced skill and reflects the flexibility of the motor memory, with greater flexibility indicating a higher quality of learning (Katak, Sullivan, Fisher, Knowlton & Winstein, 2011; Katak & Winstein, 2012; Schmidt & Lee, 2004; Wulf, 2013).

The time interval between the practice sessions and the retention test is critical and depending on the duration of this interval retention tests can be classified as immediate or delayed (Katak & Winstein, 2012). Immediate retention tests have been used anywhere between directly following the end of practice to 20 minutes later (Brydges, Carnahan,

Backstein & Dubrowski, 2007; Ishikura, 2008; Maslovat, Brunke, Chua & Franks, 2009; Winstein, Pohl & Lewthwaite, 1994), whereas delayed retention tests have been used over time periods ranging from 24 hours to two weeks (Granda Vera & Montilla, 2003; Liu & Wrisberg 2005; Sidaway, Ahn, Boldeau, Griffin, Noyes, & Pelletier, 2008; Wierink, Puttemans, Swinnen & van Steenberghe, 2005). A recent review of studies using immediate and delayed retention test has shown that 63% of the 41 studies examined found different results at immediate and delayed retention tests (Kantak & Winstein, 2012). If the aim of an intervention is to induce a learning effect, or a relatively permanent improvement in performance, then it is crucial that the retention interval is long enough to reflect permanent changes. The consolidation of the new skill within the motor memory is expected to become more stable over time (Kantak & Winstein, 2012; Krakauer & Shadmehr, 2006; Robertson, Pascual-Leone & Miall, 2004), and may be reflected in improved performance or increased resistance to interference (e.g., reduced dual task effects) (Robertson, 2004; Robertson & Cohen, 2006; Robertson, Pascual-Leone & Miall, 2004).

## **2.2 Attention**

Attention in the simplest definition of the term is used to describe the level of arousal or wakefulness within the brain (Purves, Cabezza, Huettel, LaBar, Platt & Woldorf, 2013), but the term attention is also used to refer to the level of cognitive effort that is put into completing an activity (Magill & Anderson, 2014). Attention is inherently selective in nature, and resources are allocated to process certain stimuli often at the expense of other concurrent stimuli (Purves et al., 2013).

Attention is a cognitive control mechanism that is used to manage the limited cognitive processing capacity within the brain (Pashler, 1998). The terms top down control or bottom up control are used to describe the allocation of attention to stimuli (Katsuki & Constantinidis, 2014), and attention is the mechanism responsible for identifying relevant sensory information from the environment (Johnson & Zatorre, 2006) and selecting stimuli for awareness or further processing (Pashler, 1994b). Bottom up attention refers to attentional focus that is purely driven by relevant or salient stimuli within the environment whereas, top down attention refers to attentional focus that is guided by the intentions, goals or strategies of the individual (Karatekin, 2004; Katsuki & Constantinidis, 2014). When we consider the effects of attention on motor skill performance, we are primarily

focussing on top down attentional control, although the presence of a dual task may trigger a greater involvement of bottom up attentional processes (Gazes et al., 2010).

### **2.2.1 The role of attentional focus in novel skill performance**

Attention in terms of skill performance can be divided into two types of focus: *internal* and *external* (Wulf, 2013). Internal focus is when attention is directed inwards towards the performer's body movement whereas external focus is when attention is directed outwards towards the effect the movement has had on the environment (Wulf, Höß & Prinz, 1998). A performer's focus of attention can have a significant influence on performance of a skill and an external focus of attention has been shown to facilitate both skill performance and learning (Wulf, 2013; Wulf & Shea, 2002). A lack of attention to the internal mechanics of the movement has often been associated with optimal skill performance (Beilock, Wierenga & Carr, 2002). Whilst attention to the individual skill components can in some cases be beneficial to novice performers it is usually detrimental to the performance of experts (Beilock, Wierenga & Carr, 2002).

The control of attentional focus is particularly crucial in the presence of distractions, as without an active maintenance of attentional focus incorrect responses may be selected (Kane & Engle, 2002). Experts generally have a better capability to maintain this focus than novices and sometimes even benefit from a higher cognitive load (Beilock, Wierenga & Carr, 2002; Gabbett, Wake & Abernathy, 2011; Schaefer, 2014). Furthermore, it has been shown that splitting attention between stimulus modalities does not necessarily lead to performance detriments when compared to selectively attending to one stimulus and ignoring the others (Johnson & Zatorre, 2006).

### **2.2.2 The role of attentional focus in novel skill learning**

Skilled behaviour, or motor learning is characterised by the requirement of less attentional resources than are required in the early stages of skill acquisition (Houwink et al., 2013; Taatgen, 2005). Attention is closely linked with task automaticity, specifically a task is said to be automatic when there is a reduced requirement of attentional resources (Pashler, 1994b). Therefore, an understanding of the role of attention is crucial to enhancing skill learning.

Much of the research regarding the role of focus of attention on skill learning has examined the effect of an internal or external focus of attention on balance tasks (e.g.,

Chiviakowsky, Wulf & Wally, 2010; Jackson & Holmes, 2011; Laufer, Rotem-Lehrer, Ronen, Khayutin & Rozenburg, 2007; Wulf, Höß & Prinz, 1998; Wulf, Landers, Lewthwaite & Töllner, 2009). In these (and multiple other) studies balance has been shown to be consistently improved by directing attention to markers placed on the surface the performer is balanced on as opposed to focussing on the movement of the feet (Wulf, 2013).

An external focus of attention has also been examined with regards to skill accuracy. These studies have demonstrated a beneficial effect of external attentional focus on the accuracy of various targeting skills including dart throwing (Lohse, Sherwood & Healy, 2010; Marchant, Clough & Crawshaw, 2007; Marchant, Clough, Crawshaw & Levy, 2009), golf putting (Poolton, Maxwell & Masters, 2006), a basketball free throw (Al-Abood, Bennet, Hernandez, Ashford & Davids, 2002; Zachary, Wulf, Mercer & Bezodis, 2005), volleyball serving (Wulf, McConnel, Gärtner & Schwarz, 2002), and football throw ins (Wulf, Chiviakowsky, Schiller & Ávila, 2010). Although performance improvements in external focus of attention research are generally only observed in novice performers, an improvement in skill accuracy has been found in expert performers when external focus conditions are compared to internal focus or control conditions (Bell & Hardy, 2009; Wulf & Su, 2007). Moreover, interventions that use gaze training to create an external focus of attention have shown benefits for both novice and expert performers (Vine, Moore & Wilson, 2011; Vine, Moore & Wilson, 2014)

In addition to the positive effects on skilled tasks, an external focus of attention has been shown to improve movement efficiency (Wulf, 2013). These studies have shown benefits to the use of an external focus of attention on a number of factors relevant to physical movement including maximum force production, speed and muscle activity, and isometric and isokinetic force production (Lohse, 2012; Lohse, Sherwood & Healy, 2011; Marchant, Grieg & Scott, 2009), a jump and reach task (Wulf & Dufek, 2009; Wulf, Dufek, Lozano & Pettigrew, 2010; Wulf, Zachary, Granados & Dufek, 2007), and a standing long jump (Ducharme, Wu, Lim, Porter & Geraldo, 2016; Porter, Anton & Wu, 2012; Porter, Ostrowski, Nolan & Wu, 2010; Wu, Porter & Brown, 2012).

The benefits of an external focus of attention appear to lead to relatively permanent changes in performance (Wulf, 2013), as the performance gains have been seen when the performers are asked to complete the practiced skill in novel conditions (a transfer test) (Bell & Hardy, 2009; Duke, Cash & Allen, 2011; Lohse, 2012, Ong et al., 2010),

indicating that motor skill learning has occurred. The wide range of skills examined provide evidence for the strength of an external focus of attention with regards to novel skill learning, however, the exact mechanisms underlying the benefits are yet unknown (Wulf, 2013). The use of a simple dual task protocol has the potential to facilitate an external focus condition and therefore may be extremely valuable in aiding motor skill learning.

## **2.3 Dual Tasks**

Dual task interference (DTI) occurs when two cognitive tasks or a cognitive and motor task are performed simultaneously resulting in an impairment in performance of one or both tasks (Chen et al., 2013; Goh, Ewing, Marchuk, Newton & Nyangani, 2019; Karatekin, Couperus & Marcus, 2004; Leone, Feys, Moumdjian, D'Amico, Zappia & Patti, 2017). The dual task paradigm is one of the most common methods of studying divided attention in skill performance (Karatekin, 2004). The principle of DTI arises from the assumption that there is a limited processing capacity within the brain and any additional demands would therefore cause impaired processing (Pashler, 1994a). Essentially, the need to coordinate more than one task is likely to cause the requirement for additional executive processes (Strobach, Frensch & Schubert, 2012). Performers are not usually consciously aware of experiencing DTI unless tasks are particularly mentally demanding or physically incompatible, even when reduced skill performance is clearly observable (Pashler, 1994a). The most commonly held view on DTI is that people share limited resources between tasks, hence, performing more than one task at a time reduces the capacity for completing both tasks and consequently impairs performance (Pashler, 1994a). DTI is thought to be more pronounced when the two tasks being performed contain similar inputs or require similar responses (Navon and Miller, 1987; Pashler, 1994a).

### **2.3.1 The Psychological Refractory Period (PRP)**

One of the most commonly used experimental protocols to research DTI effects is the Psychological Refractory Period paradigm (PRP). The PRP paradigm involves manipulating the temporal relationship between two tasks by shortening or lengthening their temporal overlap (Welford, 1952; Pashler, 1994a). In the PRP paradigm the time between the presentation of the stimulus for each task known as stimulus onset asynchrony (SOA), is manipulated in order to create variations in the temporal overlap of the tasks (Huestegge & Koch, 2010) (see Figure 1). A manipulation of the SOA (shortening of the duration) tends to cause a longer response time in the second task but not in the first (Luck, 1998; Oberauer &

Kliegel, 2004; Ruthruff, Pashler & Klaassen, 2001; Tombu & Jolicœur, 2003; Welford, 1952). The use of the PRP paradigm allows for the determination of the extent to which dual task interference is caused by the modality of the stimulus, cognitive processes or response processes (Bherer et al., 2005). The PRP effect has been found even when the response modalities of the two tasks are distinct, suggesting the modality of the stimulus may be a minor factor in the presence or absence of DTI (Huestegge & Koche, 2010; Luck, 1998; Marois & Ivanov, 2005; Pashler, 1994a).

Although the PRP has been commonly used in dual task research, it has been argued that the experimental manipulation of the SOA prevents the performer from expressing the true extent of their ability to multitask (Schumacher et al., 2001). Furthermore, it has been suggested that the instructions provided in PRP experiments cause the performer to prioritise the first task presented meaning that true effects of DTI are not always observed (Meyer & Kieras, 1997a). The need to produce separate responses to each stimulus may also generate additional dual task costs on top of those created by the response selection bottleneck (De Jong, 1993). When investigating situations where two tasks are presented at the same time (e.g., continuous tasks), performance in single task trials is used rather than stimulus asynchrony (Navon & Miller, 1987).

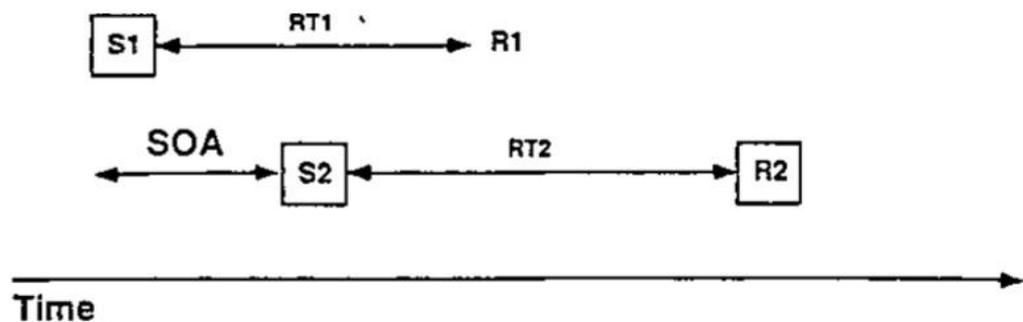


Figure 1: An example of the PRP effect. *Note:* S = stimulus, R = response, RT = response time, SOA = stimulus onset asynchrony (image from Pashler, 1994a, p.222)

### 2.3.2 Theories of dual task interference

Theories of DTI can generally be divided into two areas, those attributing the interference to bottlenecks in processing and those attributing the interference to lack of ability to share cognitive capacity (Nijboer, Taatgen, Brands, Borst & van Rijn, 2013).

### *Bottleneck models*

The cognitive system within the human brain has been described as having a 'bottleneck' for cognitive processes, which prevents the simultaneous completion of more than one cognitive task (Byre & Anderson, 2001; Pashler, 1994 a,b; Welford, 1952). However, these theories differ with regards to where the bottleneck is located, with some stating the bottleneck occurs at the response selection stage and others citing the response execution stage as the location of the bottleneck (Borst, Taatgen & Van Rijn, 2010; Bratzke, Rolke & Ulrich, 2009; Marti, Sigman & Dehaene, 2012; Navon & Miller, 1987; Pashler, 1994a). Pashler (1994b) argued that bottleneck can occur either before or after the stimuli appear, but the interference is different in nature.

The PRP effect (as discussed in section 2.3.1) is generally explained in terms of a 'bottleneck' in the response selection phase of cognitive processing which is the stage associated with the translation of the stimulus into the response (Hommel & Eglau, 2002; Oberauer & Kliegel, 2004; Pashler, 1994a,b). The response bottleneck model was developed from the information processing model of a single channel of limited capacity proposed by Broadbent (1958), and states that only one response to a stimulus can be selected at a time, and therefore the response to a second stimulus cannot take place until response selection for the first task is complete (Bratzke, Rolke & Ulrich, 2009; De Jong, 1993; Fagot & Pashler, 1992; Luck, 1998; McCann & Johnston, 1992; Pashler, 1994a,b). When the 'bottleneck' occurs performance of one or both tasks will become impaired (Pashler, 1994a). Essentially, if the response for a secondary task cannot be processed until the primary task response has been executed, there will be a delay in responding to secondary task stimuli (Schumacher et al., 2001). Suggestions of two bottlenecks, one concerned with the selection of stimuli for further processing and one concerned with converting stimulus information into a response, have also received support in the literature (Hommel & Eglau, 2002). The bottleneck model predicts that the longest response times (RTs) in a dual task condition will be observed when both tasks show similar RTs within the single task condition, leading to a higher temporal overlap (Huestegge & Koche, 2010).

Another potential cause of DTI within the bottleneck model is the structural interference effect, which might occur if both tasks require the same response modality, but the response channel can only be accessed by one task at a time (Pashler, 1994a). The greater the degree of task overlap, the more challenging it is to perform the tasks concurrently (Janssen & Brumby, 2015). In this situation if one of the tasks was continuous,

this task would need to be halted in order to produce the response to the second task (Hiraga et al., 2009). Halting of continuous tasks in dual task situations is not generally observed (Temprado et al., 2001), however, if the interference was intermittent it may be characterised by reduced performance in the continuous task rather than a complete halt (Pashler, 1994b). Whilst the presence of some form of bottleneck is generally accepted there is no consensus as to whether this bottleneck is functional or structural (Hommel & Eglau, 2002). The functional approach suggests that the capacity and resources available govern how much information can be processed at one time (Karatekin, Couperus & Marcus, 2004; Logan & Gordon, 2001; Meyer & Kieras, 1997a,b), whereas the structural approach cites that there are some limitations that are fixed regardless of experimental manipulations (Janssen & Brumby, 2015).

#### *Outcome conflicts model*

Navon & Miller (1987) proposed an alternative form of structural interference termed outcome conflict. In this theory it is proposed that DTI occurs because the response to one stimulus interferes with the processing of the second stimulus, in other words the state of some process necessary for performance of the second task is altered (Navon & Miller, 1987). Thus, the source of DTI is the inability of the processing systems within the brain to separate one task from the other or alternatively due to an attempt to avoid any potential conflicts (Navon & Miller, 1987). In this model even if the outcome of one task does not directly impede the outcome of another, interference between the two tasks can still occur as activity in one region of the brain may interfere with another region (Navon & Miller, 1987). The outcome conflict that occurs may either reduce task performance of both tasks or, alternatively cause tasks to be processed in serial to avoid performance degradation (Navon & Miller, 1987; Navon & Miller, 2002). This explanation may be dependent on the regional similarity of the neurological resources required to complete each task (Temprado et al., 2001).

#### *Adaptive Executive Control model*

An alternative theory to the central bottleneck account was proposed by Meyer & Kieras (1997a,b) who disputed the presence of central limitations to dual task processing. They developed a computational model to account for multitasking interference. The adaptive executive control model proposes that all central resources can be shared by two tasks (e.g., procedural or declarative memory) but peripheral resources (e.g., vision or motor ability) cannot be shared (Meyer & Kieras, 1997a). Within the parameters of this model,

after the conversion of the task from declarative (verbal explanations) to procedural knowledge, parallel processing of two tasks simultaneously is possible (Schumacher et al., 2001). Performance under dual task conditions is said to be accomplished by a decision rule system (Nijboer et al., 2013), and the theoretical stance of this model is that reduced performance in dual task conditions is due to either interference from common task processes, inexperience of combining the tasks or task instructions which prohibit simultaneous task processing (Meyer & Kieras, 1997a,b; Oberauer & Kliegel, 2004).

One of the aspects of this theory that is at odds with the evidence base in the literature is the task instruction element, as it has been demonstrated that DTI is still present within PRP experiments even when task priority instructions are removed (Carrier & Pashler, 1995; Pashler, 1994; Pashler, Carrier & Hoffman, 1993; Ruthruff, Pashler & Klaassen, 2002). The advantage of 'executive control' theories is that they allow for the reduction of dual task interference with experience (Schumacher et al., 2001). Although this model proposes that dual processing is possible it also suggests that bottlenecks can occur at any stage in task processing and therefore it is still essentially a bottleneck model (Tombu & Jolicœur, 2003). This theory was developed further into the Executive Process Interactive Control (EPIC) model proposed by Kieras & Meyer (1997c) which talks about the theory of parallelism in terms of the stage of skill learning and suggests that parallel processing should be avoided early in the skill learning process.

#### *Capacity sharing models*

One of the earliest explanations for DTI was that of capacity sharing which describes the PRP effect from a perspective of shared processing capacity (McLeod, 1997; Navon & Miller, 2002; Tombu & Jolicœur, 2003). This group of theories is based on the premise that the stimulus processing capacity must be shared as resources are limited, and according to this principle as the SOA between the two tasks gets shorter the amount of time the tasks must spend sharing the resources is extended (Tombu & Jolicœur, 2003), although this may only apply to central operations and not to all aspects of task processing (Posner & Boies, 1971). While theories of central capacity sharing argue that resources can be shared by more than one task, they also note that performance degrades when there is a limited capacity of a single resource and thus performance quality is determined by the characteristics of the individual tasks (Navon & Gopher, 1979; Tombu & Jolicœur, 2003).

Some central capacity models of dual task interference (Meyer & Kieras, 1997a,b; Navon & Miller, 2002; Tombu & Jolicœur, 2003) indicate that under certain conditions the

allocation of attentional resources is uneven, for example, when one task is presented before another, or when one task has particularly high attentional demands (Navon & Miller, 2002). Whilst early researchers suggested that the level of DTI may be influenced by the modality of the tasks, indicating that tasks requiring different response modalities require less attentional resources (Wickens, 1991), this has been disproven by a number of studies which have shown that DTI is present even when a combination of visual and audio modalities were used (Eimer, Van Velzen & Driver, 2002; Gherri & Eimer, 2011; Kunar, Carter, Cohen & Horowitz, 2008; Spence Pavini and Driver, 2000; Strayer & Johnston, 2001).

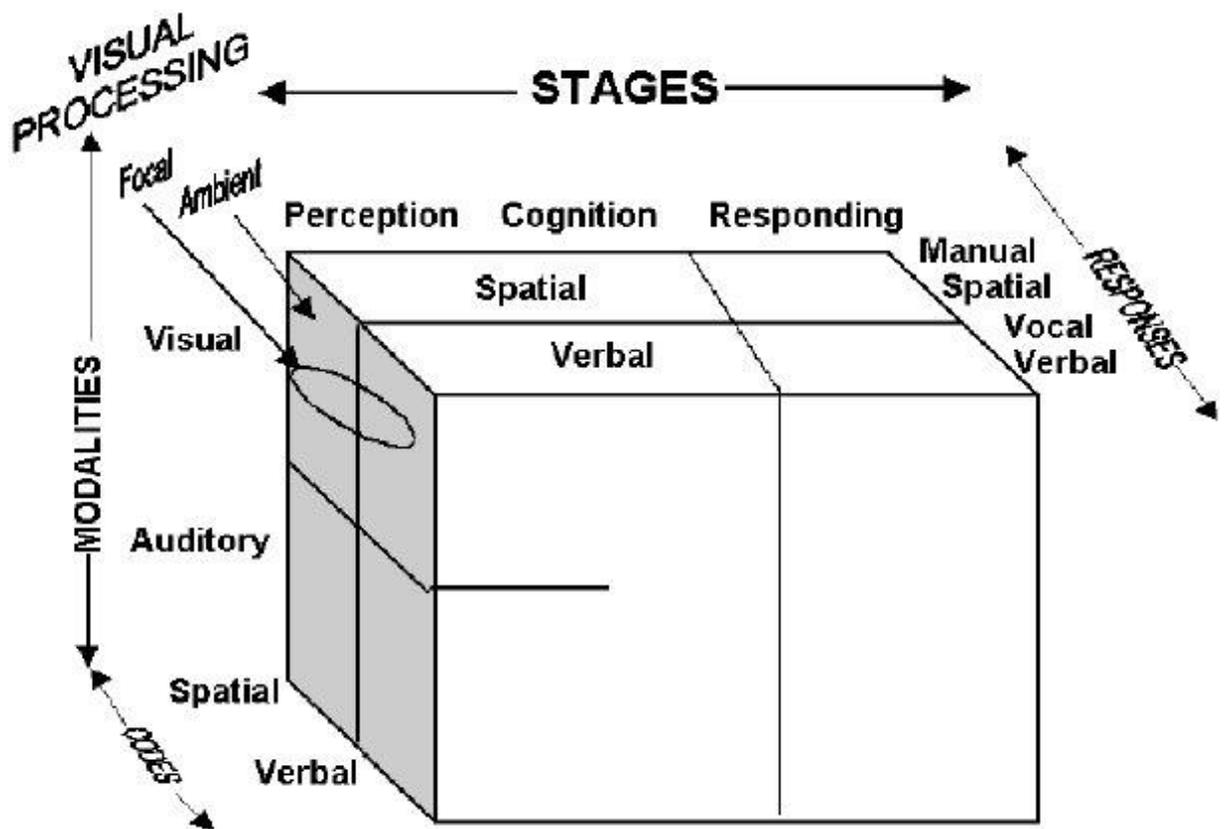


Figure 2: The 4-D multiple resource theory (image from Wickens, 2008, p. 450)

#### *The multiple resource models*

An extension of the capacity sharing models are the multiple resource theories (Navon & Gopher, 1979, Wickens, 2002, 2008). These theories are based on the principle that a task may require multiple resources, and the extent to which tasks require similar resources will dictate the extent to which tasks interfere with each other (see Figure 2). According to Wickens (2002), the more resources that are shared, the more interference increases.

Therefore, tasks which have no common processes will interfere with each other to a lower level than would be expected by their individual task demands alone (McLeod, 1977). The possibility of completing more than one task in parallel with no adverse effects exists within the confines of this theoretical approach provided the two tasks do not require the same resources (Schumacher et al., 2001). This theory gives an indication of the amount of interference we can expect to see but does not clarify the source of the interference (Nijboer et al., 2013).

Whilst a number of authors have described mechanisms by which two tasks can be completed in parallel (Meyer & Kieras, 1997a,b; Navon & Miller, 2002; Tombu & Jollicer, 2003; Oberauer & Kliegel, 2004), the extent to which this parallelism can occur has often been debated in the literature (Taatgen, 2005). A series of experiments using a paradigm similar to PRP have demonstrated that two tasks presented in close succession can have simultaneous access to the same stimulus response network as long as no conflict exists between the two tasks (Fisher, Miller, Schubert, 2007; Logan & Shukind, 2000). This finding of a shared stimulus response network is supported by the work of Allport, Antonis & Reynolds (1972) who proposed that if the two tasks did not require any of the same processors they could be performed in parallel.

#### *Threaded cognition model*

The most recent theory of dual task interference is the theory of threaded cognition which was developed by Salvucci & Taatgen (2008). This theory suggests that cognitive resources can operate in parallel, via a mechanism that can provide conflict resolution but allows for concurrent task processing (Liu, Feyen, Tsimhini, 2005; Salvuci & Taatgen, 2008; Wu & Liu, 2007). The theoretical basis of this concurrent processing is that cognitive processes are represented by 'threads' that are coordinated by a single cognitive processor that not only combines inputs from sensory or motor regions but also initiates the processing required (Salvucci & Taatgen, 2008). A thread is defined as 'all processing in service of a particular goal, including procedural processing' (Salvucci & Taatgen, 2008, p.107). Although parallel processing can occur each resource can only be used by one process at a time (Salvucci & Taatgen, 2008; Taatgen et al., 2009). Consequently, interference occurs when a task requires a resource that is currently in use by another task (Nijboer et al., 2013). If a resource is available and a particular 'thread' has need of it, it will take the resource and as soon it is no longer required it will be released (Taatgen et al., 2009) (see Figure 3). Furthermore, if the interval between two tasks is particularly short a phenomenon called an

*attentional blink* can occur impairing response to the second task (Taatgen et al., 2009), and the threaded cognition model of dual task interference attributes this attentional blink to a state of excessive cognitive control (Taatgen et al., 2009). The threaded cognition theory is in line with findings that have demonstrated both serial and parallel task processing components (Nijboer et al., 2013; Sigman & Dehaene, 2008) but it is not as widely accepted as the bottleneck and central capacity models.

In addition to these theories DTI effects can also be interpreted in terms of a fixed capacity resource model of attention (Hiraga, Garry, Carson & Summers, 2009). This model states that when the resources required to complete two tasks exceeds the resources available the amount of resources allocated to one or both tasks will be reduced leading to impaired performance in one or both tasks (Kahneman, 1973). Another approach to this is to consider the amount of attentional resources to be of less importance than how these resources are allocated (Karatekin, Couperus & Marcus, 2004). Therefore, an emphasis is placed on top down attentional control and attention is viewed as skill rather than a resource (Hirst & Kalmar, 1987; Lavie, Hirst, De Fockert & Viding, 2004; Meyer & Kieras, 1997a). From this perspective the ability to perform two tasks simultaneously could be

trained and improved.

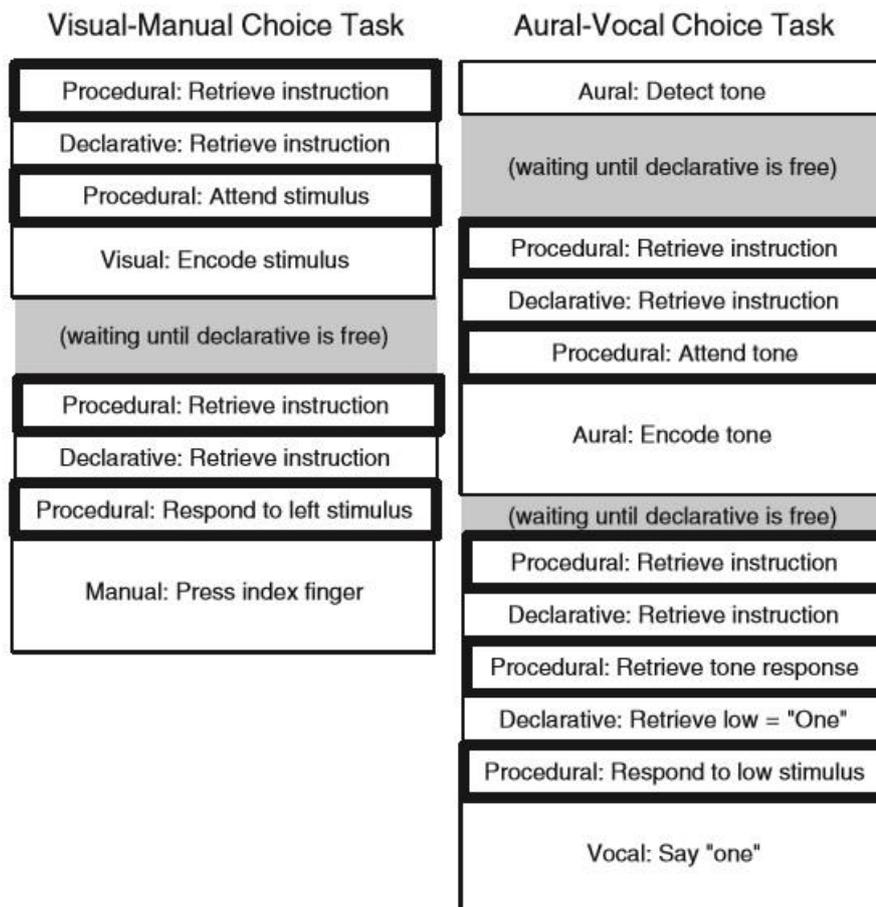


Figure 3: An example of dual task behaviour in a novel task under the theoretical framework of Threaded Cognition Theory (image from Salvucci & Taatgen, 2008, p.114).

Whilst the individual theories differ considerably in their explanations for the mechanisms behind DTI, they are not necessarily mutually exclusive and different theoretical accounts may be applicable for different types of tasks (Pashler, 1994a). There is not yet any universal explanation to predict the costs of a dual task in any given situation (Schaefer, 2014).

### 2.3.3 Dual tasks and skill performance

Substantial differences in the ability to coordinate two tasks have been observed between individuals (Bherer et al., 2005). DTI occurs even when both tasks are extremely easy or distinctly different (Pashler, 1994b; Pellecchia, 2005). Dual task performance is not the same across individuals and is influenced by factors such as age and experience level. The age

effect has been extensively investigated, with older individuals generally experiencing greater dual task effects (Bherer et al., 2005; Hartley, 2001; Kramer, Larish & Strayer, 1995; Verhaeghen & Cerella, 2002; Verhaeghen, Steitz, Sliwinski & Cerella, 2003). Task experience is another important factor which governs the extent of DTI with novices generally performing worse in dual task conditions, while individuals who are well practiced or experts in the skill being examined are usually able to complete simultaneous tasks with no negative effect on performance (Beilock et al., 2002; Beilock, Wieringa & Carr, 2002; Oberauer & Kliegel, 2004). It has been suggested that novices generally benefit from skill focussed conditions (Schaefer, 2014), although performance is usually enhanced by focussing attention to the external aspects of the skill rather than the internal movement characteristics. Therefore, the effect of dual tasks on novel skill performance could be seen to arise from the increased cognitive demands of a dual task rather than from a change in attentional focus.

Some individuals appear to be immune to dual task costs with around 2.5% of the population being characterised as 'supertaskers', or individuals who are not affected by dual task costs (Donohue, James, Eslick & Miroff, 2012; Medeiros-Ward, Watson & Strayer, 2015; Watson & Strayer, 2010). Furthermore, people who are better able to perform individual tasks are generally more immune to DTI when a secondary task is introduced (Janssen & Brumby, 2015) and it has been suggested that there is no such thing as a 'hard-wired' limit to dual task capacity (Oberauer & Kliegel, 2004). One interesting finding in the literature in relation to dual task performance is the fact that an element of DTI is observed, not only when the dual task is present, but also in response to an expectation of the task even if it does not appear (Gottsdanker, 1979; Logan & Gordon, 2001; Navon & Gopher, 1979). This finding indicates a potential involvement of an anticipatory mechanism in the occurrence or magnitude of DTI.

The role of dual task characteristics in DTI is unclear, whilst some authors have indicated that dual task complexity has a significant effect on the amount of DTI observed with continuous tasks generally thought to be complex than discrete tasks (Gopher, Brickner & Navon, 1982; Maynard & Havel, 1987; Oberauer & Kliegel, 2004; Rice et al., 2012; Ruthruff, Pashler & Klaassen, 2001). However, it has also been shown that single task complexity does not determine dual task interference (Navon & Millier, 1987). We might assume that if the response to one task is expected to be slower than processing the response to the 'quicker' task may take priority if no instructions are given (Huestegge & Koche, 2010). In reality during a PRP paradigm in particular, unless instructions are

provided to the contrary, priority tends to go to the task that is presented first, thus the longer response time in the second task would be strategic rather than structural (Koch, 2009; Meyer & Kieras, 1997a,b; Navon & Miller, 2002; Ruthruff, Miller & Lachmann, 1995; Tombu & Jollicer, 2003). While there is debate in the literature with regards to whether this strategic prioritisation is possible, it has been demonstrated that when no priority instructions are given people are able to adapt their strategy to prioritise one task over another in order to meet performance objectives, indicating that attentional allocation can be strategically adapted (Janssen & Brumby, 2010). In order to limit the influence of strategic prioritisation it is important that no instructions about task priority are given (Ruthruff, Pashler & Klaassen, 2001). Despite this evidence in support of the possibility of strategically prioritising one task over another, there is no evidence that it is possible to choose to complete both tasks at the same time but slower (Pashler, 1994b).

There are some instances when DTI is not observed and this has been explained by a phenomena called a latent bottleneck (Ruthruff, Johnston, Van Selst, Whitsell & Remington, 2003), which refers to the assumption that the temporal overlap between the tasks is so high that the response to the first task has already been completed before the second task is presented (Huestegge & Koche, 2010). An alternative explanation for this effect can be found in the Yerkes-Dodson law (Yerkes & Dodson, 1908), whereby the presence of a relatively simple dual task, rather than interfering with the primary task performance, can create the perfect level of activation, motivation and concentration (Curran & Stokes, 2003). Furthermore, it is likely that there are individual differences in capacity to maintain attentional focus (Kane & Engle, 2002). The failure of some studies to show DTI may simply be a function of methodological design. A study by Friedrich, Scherer, Sonnleitner & Neuper (2011) found that the presence of a passive audio task, did not impair primary task performance. However, just the presence of an audio tone which required no response would not normally be expected to impair performance as the key determinant of DTI appears to be a requirement to produce a response (Pashler, 1994a,b).

While the majority of literature discusses dual task effects in terms of magnitude of effect, or the presence or absence of DTI, an alternative finding has been reported, suggesting that the presence of dual task interference is not just dependent on expertise but also on task type. Hemond, Brown and Robertson (2010) conducted a study examining the performance of a novel motor sequencing task whilst completing a secondary task. Hemond, Brown and Robertson (2010) observed that whilst counting the number of red cues impaired performance of the sequence task, concurrently learning a second sequence

significantly enhanced performance of the motor sequence task. They concluded that the completion of the two sequencing tasks engaged similar neural networks and therefore enabled the availability of a greater amount of neural resources for completion of the primary task. It is not yet clear whether this enhanced dual task performance is specific to the tasks used in this study or whether it would be transferable to other task combinations.

#### **2.3.4 Training of dual tasking ability**

The DTI effect is not fixed and can be reduced or eliminated with practice. One of the earliest studies to discuss the elimination of DTI with practice was that of Schumacher (2001) who demonstrated that with 2000 trials of practice, dual task costs with regards to response time could be eliminated although dual task costs were still seen in relation to errors. Dual task training effects were also observed by Hazeltine, Teague & Ivry (2002) who were able to eliminate DTI effects on both response times and errors. These findings were contradicted in a series of experiments by Levy & Pashler (2001), whose findings indicated that the elimination of DTI was only possible when a specific combination of tasks was used that did not utilise the same response modalities, for example a combination of visual and manual tasks.

The possibility of combining the two tasks with almost complete time sharing exists after just a small amount of practice, however, cautious executive processing may cause the postponement of one task which provides an explanation for the individual differences in DTI effects (Schumacher et al., 2001). Ruthruff and colleagues (2006) gave participants eight dual task training sessions and observed a reduction in DTI, although interference was not completely eliminated. A more recent study, however, demonstrated that dual task interference was eliminated after 50 practice trials (Schaefer & Lang, 2012) and these findings were supported by the work of Pellecchia (2005) who found that three sessions of dual task training were sufficient to eliminate the dual task effect. Further to this, Worden & Vallis (2014) found that concurrently practicing a motor and cognitive task enhanced the performance of both tasks, however, the dual task practice group received additional trials to the single-task practice group and consequently these results must be regarded with caution. One important factor to note when considering training of dual task ability is that dual task practice is only effective in reducing or eliminating DTI when both tasks are practiced together, not when the two single tasks are practised in isolation (Oberauer & Kliegel, 2004).

The findings of the elimination of DTI following practice challenges the presence of a central bottleneck and indicates that, whilst a bottleneck may be present when a task is novel, it is not a structural feature of the cognitive processing systems (Oberauer & Kliegel, 2004; Pashler, Johnston & Ruthruff, 2001). An alternative argument that has been presented is that whilst dual task interference can be reduced with practice it is not possible to fully eliminate it (Brown & Bennett, 2002; Ruthruff et al., 2006). Ruthruff et al. (2006) suggested that reduced interference after practice was not due to the elimination of the bottleneck in processing, but rather, a reduction in the duration of this bottleneck. Resource models of dual task effects (Navon & Miller, 2002; Tombu & Jolicoeur, 2003), do provide an explanation as to why DTI disappears after practice by proposing that following extensive practice the demand for resources to complete the two tasks would theoretically be reduced. Another explanation is that extended dual task practice serves to integrate the two individual tasks into one which may be possible when there are limited stimuli and response combinations (Ruthruff et al., 2006; Oberauer & Kliegel, 2004; Schumacher, 2001). Dual task training may serve to automatise one or both tasks and therefore reduce attentional demands, or alternatively that by practicing two tasks concurrently more efficient time sharing between the tasks is developed (Brown & Bennett, 2002). Furthermore, dual task training may not only improve the tasks being trained but also create a set of skills which facilitates performance in other dual task conditions (Bherer et al., 2005; Erickson et al., 2007)

One interesting aspect of dual task practice is the fact that, while older adults are more affected by dual task interference this effect can be greatly reduced by dual task training, which indicates a plasticity in cognitive processes (Bherer et al., 2005; Pellecchia, 2005). In fact, older adults appear to be more responsive to dual task training than younger adults (Bherer et al., 2005). Taken together the findings that DTI can be reduced or eliminated in different populations indicate that the dual task interference effect is not a fixed phenomenon and can be altered by training.

### **2.3.5 The influence of dual tasks on skill learning**

Due to the negative effects of dual task interference on performance of a novel task it would be expected that learning would be impaired under dual task conditions. In the early stages of skill acquisition any additional demands that are placed on the attentional system (such as a dual task) may impair learning (Nissen & Bullemer, 1987; Rémy, Wenderoth, Lipkens & Swinnen, 2010; Shanks & Channon, 2002), but as the task is learnt the

interference becomes either low or non-existent (Rémy et al., 2010). It is thought that DTI in the early stages of learning comes from competition for the same neurological resources (Rémy et al., 2010). Learning under dual task conditions has been shown to be smaller than under single task conditions (Frensch, Wenke & Rüniger, 1999). However, other studies have shown no effect of dual tasks on skill learning when compared to learning under single task conditions (Jiménez & Méndez, 1999, 2001), and the mechanisms of implicit skill learning are observed independent of task load (Jiménez & Vázquez, 2005). This lack of effect of dual tasks may be due to a lack of complexity in the primary task. Elion, Sela, Bahat, Siev-Ner, Weiss and Karni (2015) found that training in dual task conditions neither impaired nor facilitated learning, however, the primary task used was a simple balance task which may not have required any extensive training to develop.

### **2.3.6 Beneficial effects of dual tasks on skill learning**

Whilst training under dual task conditions is more demanding and often results in initial performance decrements, it appears to make people more responsive to training (Erickson et al., 2007; Malone & Bastien, 2010). The decrease in skill performance is generally observed during the early stages of dual task training but not in later stages (Beilock et al., 2002; Rémy et al., 2010). Furthermore, training in dual task conditions produces longer lasting effects than training in single task conditions (Malone & Bastien, 2010), with better performance seen during skill retention tests following dual task training compared to single task training (Chiou & Chang, 2016; Roche et al., 2007).

There appears to be a relationship between the level of impairment during training and the amount of improvement in skill retention tests. Roche et al., (2007) found that the longer the response time in the training block, the quicker the response time in the retention task, The finding of initial decreases in performance but longer term improvements is in line with motor learning literature which suggests a more demanding training environment (such as the use of contextual interference) promotes longer lasting gains in skill acquisition (Porter & Magill, 2010; Wright, Verwey, Buchanen, Chen, Rhee & Immink, 2016). However, following a period of training in dual task conditions removal of that dual task has been shown to produce a decrease in learning (Schmidtke & Heuer, 1997). This finding is supported by Song & Bédard (2015) who demonstrated that dual task conditions did not impair learning, however, this was only evident when a dual task was also present at retention. One particularly interesting finding of this study is that the dual task present at retention did not need to be the same as the one used during training, the

presence of alternative dual tasks was sufficient to trigger the improved performance (Song & Bédard, 2015).

The facilitation of learning in dual task training occurs even when the primary and secondary tasks have very different mechanisms (Brown & Bennett, 2002). However, facilitation of learning effects is task dependent, with not all secondary tasks eliciting benefits (Roche et al., 2007; Goh et al., 2012). The exact nature of the task required to elicit learning benefits has not yet been determined. The work of Roche et al. (2007) demonstrated that secondary tasks with greater attentional demands (greater complexity) produced more facilitation to primary task performance than those with lesser demands.

Goh et al. (2012), however, found that the relationship of the complexity of the dual task to the learning gains was dependent on the time of task presentation, with a more complex (choice response) task being beneficial to learning when presented during the preparation phase of the movement but detrimental to learning when presented during the execution phase of the movement. For a less complex dual task (simple response) however, the opposite findings were observed, with learning enhanced when the task was presented during the execution phase of the movement but not when it was presented during the preparation phase of the movement (Goh et al., 2012). Whilst the mechanism behind the improvements observed has not been identified, the presence of a dual task during novel skill learning may cause a greater investment in cognitive encoding during the early stages, which may in turn facilitate the development of a stronger long term memory representation (Kantak & Winstein, 2012). Moreover, learning in dual task conditions may improve primary task automatization (Clark, 2015; Ruthruff et al., 2006), and consequently reduce the cognitive capacity required for the primary task (Mibs, Elsner & Hofheinz, 2016). In addition, training in dual task conditions may also aid learning by improving informational processing speed (Ruthruff et al., 2006) meaning that performers are more able to process environmental or feedback cues efficiently.

An alternative explanation for dual task benefits to skill learning is that practice in dual task conditions may improve learning by creating an implicit learning environment. According to theories of skill learning it is generally accepted that the process of acquiring a skill initiates in a cognitive phase and following practice becomes more autonomous (e.g. Fitts & Posner, 1967). Learning a skill in an implicit manner enables the learner to 'skip' the cognitive stage of learning and quickly progress to more autonomous processes (Masters and Poolton, 2012). Masters (1992) used a dual task condition to reduce the involvement of

explicit processes in the initial stages of skill learning and consequently to promote the use of implicit processes. This study found that although learning was impaired in the dual task group compared to the explicit learning group, training in dual task conditions did reduce skill breakdown under pressure, indicating that more autonomous processes were being employed by this group.

Subsequent studies have provided support for this finding demonstrating that learning skills in implicit conditions promotes resistance to breakdown of skilled performance under pressure (Lam, Maxwell & Masters, 2009; Liao & Masters, 2001). However, despite improved resistance to pressure the rate of learning in dual task conditions has been shown to be slower than in single task conditions (Masters, 1992) which suggests that the use of implicit learning techniques cannot explain the improved rate of learning observed in some dual task studies.

Roche et al. (2007) explained the dual task benefits to skill learning observed in their study using an attentional focus perspective. Their study utilised a simple secondary task that did not require executive processing, meaning that minimal attentional resources would be required to perform the task. Roche et al. (2007) proposed that this simple secondary task may have served to raise arousal and aid attentional focus on the primary task. An alternative explanation which may support the facilitation of attentional focus is that rather than facilitating arousal the presence of a dual task promotes an external focus of attention. An external focus of attention has been shown by numerous studies to facilitate skill acquisition (see Wulf, 2013 for a review). However, in the context of these studies an external focus of attention is generally considered as a focus on the effects of the movement (e.g. Wulf, Höß & Prinz, 1998; Wulf & Su, 2007). However, dual tasks have been used to facilitate an external focus of attention both in skill learning (Beilock, Carr, MacMahon & Starkes, 2002) and postural control (Laufer, 2008). Goh and colleagues (2012) compared the explanation that dual task training serves to promote arousal and facilitate attention proposed by Roche et al. (2007), with the explanation of enhanced neurological network activation proposed by Hemond, Brown and Robertson (2010) in their study on dual task benefits to performance. Using a choice audio-response task and a simple audio-response task applied at different movement phases, Goh et al. (2012) determined that dual task benefits to motor learning were observed due to task similarity rather than facilitation of attentional focus due to enhanced arousal. The similarity based dual tasks led to enhanced learning of the primary task when compared to a dual task condition which was more complex and used to promote arousal. This study did not examine whether the

dual task facilitated an external focus of attention so this may provide an alternative explanation for the results obtained.

In recent years there has been a great deal of interest in the benefits of dual task training in older adults and clinical populations. Dual task training has been shown to improve gait, balance and walking in older adults (Azadian, Torbati, Kakhki & Farahpour, 2016; Hiyamizu et al., 2012; Silsupadol et al., 2009; Worden & Vallis, 2014), which are maintained up to 6 months following the cessation of training (Gregory et al., 2016). Conversely, learning of a cognitive task in dual task conditions has been shown to be impaired in older adults (Vandenbossche et al., 2014) indicating in this population at least that the benefits are task dependent. However, Vandenbossche et al. (2014) did find that older adults were able to learn implicitly under dual task conditions provided the secondary task shared some characteristics with the primary tasks. In addition to the benefits of dual task learning in older populations, over the last few years research into the benefits of dual tasks in clinical populations has begun to develop with enhanced recovery observed in stroke survivors who trained in dual task conditions (An et al, 2014; Choi, Lee & Lee, 2015; Kim et al., 2014; Plummer et al., 2014). Furthermore, Fritz, Cheek & Nicholas-Larson (2015) conducted a systematic review which indicated that dual task training has potential for improving balance, walking, and cognition in people with neurological disorders such as brain injury, Parkinson's disease and Dementia, although they surmised that the lack of high quality controlled studies meant that the evidence in support of this type of training for these conditions was not conclusive. The positive results observed however, indicate that dual task training has a great deal of potential to aid neurological rehabilitation.

### **2.3.7 Neural correlates of dual task interference**

Learning of a task requiring any level of executive control has been shown to result in specific changes in task related brain activity (Erickson et al., 2007) and consequently there has been a lot of interest in the literature in identifying a specific region of the brain which is responsible for DTI. Dual tasks have been shown to activate a range of different regions in the brain including the right and left inferior frontal gyri, and the dorsolateral prefrontal cortex (DLPFC) (Erickson et al., 2007). Sigman and Deheane (2008) were able to find evidence in support of parallel processing in perceptual and motor tasks using fMRI to show that the bilateral posterior parietal cortex, premotor cortex, supplementary area, anterior part of the insula and cerebellum were shared by both tasks during dual task performance.

Additional regions of the cerebellum have been shown to activate in dual task performance when compared to single task performance (Wu, Liu, Hallett, Zheng & Chan, 2013). Overlap has also been identified in the lateral frontal and parietal regions during the dual task performance of a visual and motor task (Rémy et al., 2010) and in the prefrontal cortex, inferior parietal cortex, inferior frontal sulcus, the middle frontal gyrus and the intraparietal sulcus during performance of a visual and auditory task (Collete et al., 2005; Szameitat, Schubert, Muller & Yves von Cramon, 2002). Van Impe et al. (2011) failed to find a single region responsible for dual task interference, demonstrating instead an uprating of activation in all regions associated with primary task performance.

Neural correlates of DTI have also been investigated in terms of dual task training effects. Rémy et al. (2010) found that prior to training dual task interactions occurred in the PFC, the parietal regions, the right frontal gyrus, the cerebellum and the thalamus, however, the interaction in the frontal and parietal regions were reduced in the post training session. Furthermore, Rémy et al. (2010) found that in the pre training session the dual task condition demonstrated reduced activity in all cortical areas when compared to the sum of the activity in the single task condition, although they were not able to identify a single region that was specifically recruited to complete the dual task. Goh, Lee and Fisher (2013) studied the enhanced skill performance and learning in dual task conditions and indicated that the dorsal premotor cortex was involved in enhanced motor learning in dual task studies. A recent systematic review of this area has indicated that there is no single additional area responsible for dual task interference, although prefrontal activation was consistently demonstrated to increase, indicating involvement of this region (Leone et al., 2017). It may be that a lack of a specific locus for DTI is caused by the fact that concurrent tasks are performed well enough that the resources used for the single tasks are sufficient, and no additional dual task specific resources are required (Leone et al., 2017).

The role of the PFC in DTI has been cited by a number of studies. Sigman and Dehaene, (2008) found that a PRP paradigm induced delayed activity in the PFC implying a role for this region in dual task processing and a potential involvement of this region as part of a central bottleneck. Furthermore, concurrent performance of more than one task requires attentional resources to be distributed to multiple simultaneous processing systems, which is a role primarily conducted in the PFC (Jaeggi et al., 2003). A proportional increase in PFC activation with an increase in dual task difficulty has been observed (Mirelman et al., 2014) and the PFC has shown increased activation following dual task training indicating a shift in the location of DTI to the prefrontal regions (Erikson et al.,

2007). This was also demonstrated by an earlier study that showed activation in the DLPFC in dual task conditions but not in single task conditions (D'Esposito, Detre, Alsop, Shin, Atlas & Grossman, 1995). The right DLPFC in particular has demonstrated increased activity under dual task conditions (Mandrick, Derosiere, Dray, Coulon, Micallef & Perrey, 2013), and a bilateral increase in DLPFC activation has been observed during complex but not simple dual tasks (Iidaka, Anderson, Kapur, Cabeza & Craik, 2000; Jaeggi et al., 2003). Furthermore, lesion studies have shown a substantial decrease in dual task performance in patients with prefrontal cortex damage even when single task performance is unaffected (Baddeley, Della Sala, Papagno & Spinnler, 1997).

The left DLPFC has been indicated as a region responsible for DTI (Johnson & Zatorre, 2006), however a recent study has indicated that facilitatory rTMS over the left DLPFC did not improve dual task walking (Goh et al., 2019). Other studies have also failed to show additional DLPFC activation under dual task conditions (Adcock, Constable, Gore & Goldman-Rakic, 2000; Bunge, Klingberg, Jacobsen & Gabrieli, 2000; Fletcher Shallice & Dolan, 1998; Goldberg et al., 1998; Klingberg, 1998). These studies used single tasks that engaged the prefrontal cortex and thus there is evidence that if the DLFC is already activated, presentation of a secondary task does not illicit any additional activation (Jaeggi et al., 2003; Kane & Engle, 2002), although an increase in the magnitude of the response may be observed (Adocok et al., 2000; Bunge et al., 2000; Johnson & Zatorre, 2006; Van Impe et al., 2011; Wu et al., 2011). This increase in response magnitude in dual task conditions has been demonstrated to align with the sum of activations observed in single task conditions (Jaeggi et al., 2003; Just, Carpenter, Keller, Emery, Zajac & Thulborn, 2001), although an overactivation in the DLPFC which is greater than the sum of the activation caused by the single tasks has also been demonstrated (Blumen, Holtzer, Brown, Gazes & Verghese, 2014; Leone et al., 2017; Van Impe et al., 2011; Wu et al., 2013). Thus, it appears that there are processes exclusive to the dual task condition that are activated in the prefrontal cortex such as task coordination or attentional switching (Firth & Dolan, 1996; Ingvar, 1994; Jaeggi et al., 2003; Leone et al., 2017). Furthermore, tasks that make minimal demands on the DLPFC individually such as simple responses to stimuli may cause additional activation in the DLPFC when completed together (Kane & Engle, 2002).

It has been posited that there is a limitation in task processing capacity in the PFC and consequently activation will increase until this level is reached before attenuating (Jaeggi et al., 2003). In contrast to the findings of overactivation in dual task conditions some studies have shown a reduction in activation when dual task conditions are compared

to single task conditions (Just, Kellar & Cyncar, 2008; Rémy et al., 2010). It has been suggested that this under activation may result from one task taking away resource time from another leading to a level of activity which is less than that observed in single task conditions (Leone et al., 2017).

DTI effects have been investigated using NIRS and oxygenation levels in the prefrontal cortex have been shown to increase under cognitive motor dual task conditions even when there is no decrease in primary task performance (Holtzer, Mahoney, Izzetoglu, Izzetoglu, Onaral & Verghese, 2011; Meester, Al-Yahyya, Dawes, Martin-Fagg & Piñon, 2014; Mirelman et al., 2014).

## **2.4 The prefrontal cortex (PFC)**

The functions of the prefrontal cortex (PFC) support the involvement of this region in DTI. The PFC consists of a complex region of neurological processing where individual functions are highly localised (Hoshi & Tamura, 1997), and is the region of the brain responsible for attention to action (Jueptner, Stephan, Frith, Brooks, Frackowiak & Passingham, 1997). Enhanced levels of [O<sub>2</sub>Hb] and [HHb] have been observed in response to increased attentional requirements in the PFC (Toichi et al., 2004) and increased oxygen [O<sub>2</sub>] utilisation is observed in the PFC for tasks of attention compared to tasks involved in higher cognitive processing (Toichi et al., 2004). A larger number of neurons may be activated in tasks requiring high levels of attention as the task tends to be less specific in nature (Toichi et al., 2004), with sustained attention localised to the right prefrontal cortex (De Joux, Russel & Helton, 2013; De Joux, Wilson, Russel, Finkbeiner & Helton, 2017). The right PFC also appears to be related to dual task performance (McKendrick, Ayaz, Olmstead & Parasuraman, 2014) and the dorsolateral PFC (DLPFC) plays a role in blocking the effects of distractions (Kane & Engle, 2002).

## **2.5 Near Infrared Spectroscopy (NIRS)**

Near infrared spectroscopy (NIRS) utilises the transmission of near infrared light through biological tissue to collect non-invasive, in vivo measurements of haemodynamic changes within the region of interest (Ehlis, Herrmann, Wagener & Fallgatter, 2005; Tamura, Hoshi & Okada, 1997; Vinette, Dunn, Slode & Federico, 2015). When photons pass through biological tissue the nature of their transmission is dependent on the reflectance, scattering and absorption properties of the biological material (Jobsis, 1977). NIRS is based on the intrinsic optical absorption properties of the chromophores within blood (Huppert, Hoge,

Diamond, Francheschini & Boas, 2006). NIRS devices capitalise on the wave length dependent absorption and scattering properties of light within the infrared spectrum to quantify the concentrations of oxyhaemoglobin [O<sub>2</sub>Hb] and deoxyhaemoglobin [HHb] within the microvasculature (Delpy & Cope, 1997; Jobsis, 1977; Selb, Boas, Chan, Evans, Buckley & Capr, 2014; Schreppel et al., 2008). Because of the relative transparency of biological tissue, light within the near infrared spectrum (700-1000nm) is able to propagate several centimetres below the device emitter (Boas, Elwell, Ferrari & Taga, 2014; Ehlis et al., 2005; Hoshi, 2003; Pellicer & del Carmen-Bravo, 2011).

The chromophores [O<sub>2</sub>Hb] and [HHb] are dominant within the infrared spectrum and therefore easily identified (Gagnon et al., 2012; Strangman, Goldstein, Rauch & Stein, 2006). Moreover, the absorption pattern of [O<sub>2</sub>Hb] is different from that of [HHb] meaning that the quantities of each chromophore can easily be distinguished in this spectrum (Ferrari & Quaresima, 2012; Obrig & Villringer, 2003; Pellcier & del Carmen Bravo, 2011; Scheeren, Schober & Schwarte, 2012; Villringer & Chance, 1997). NIR light absorption by [O<sub>2</sub>Hb] is highest at 850 nm whereas absorption by [HHb] is highest at 760 nm (Bhambhani, Maikala, Farg & Rowland, 2006; Scholkmann et al., 2014). While the chromophores [O<sub>2</sub>Hb] and [HHb] both have absorption spectra within the NIR range, water which is the dominant tissue chromophore, absorbs below 300 nm and above 1000 nm (Delpy & Cope, 1997; Scholkmann et al., 2014) (see Figure 4 for light absorption spectra of [O<sub>2</sub>Hb], [HHb] and water). Between 400 and 650 nm (the visible spectrum) light is predominantly absorbed by several tissue components rather than reflected, thus the near infrared spectrum is the only region where absorption of light by the tissue is sufficiently low for returning light from the tissue to be detected (Delpy & Cope, 1997; Scheeren, Schober & Schwarte, 2012; Scholkmann et al., 2014). Using NIRS the amount of reflected (not absorbed) light returning from the tissue is detected by an optical detector probe situated 2-5 cm from the light source and the attenuation of the light is used to determine the concentration of the chromophores within the region of interest (Ehlis et al., 2005; Ferrari et al., 2014).

In the NIR light range light is less scattered and is absorbed by the chromophores [O<sub>2</sub>Hb], [HHb] and myoglobin [Mb], and the enzyme cytochrome oxidase [Cyt-Ox] which is found in mitochondria (Hoshi, 2003). The redox state of [Cyt-Ox] is an index of intracellular oxygen availability and can be determined using NIR light (Hoshi, 2003; Reynolds et al., 1988). Whilst [Cyt-Ox] measurements may provide more direct information about neural activity than haemoglobin (Heekeren et al., 1999; Jobsis, Keizer, LaManna & Rosenthal, 1977), it is not greatly discussed in the literature and consequently the validity of the use of

this measure for determining neurological activation has yet to be established. Furthermore, the Artinis Portalite NIRS device investigated in this thesis does not allow measurements of [Cyt-Ox] to be undertaken and therefore no further consideration will be given to this enzyme in relation to the determination of neurological activation. As [Mb] is found only in the muscle the absorption of light by [Mb] is unlikely to contribute significantly to cerebral measures since only minimal levels of muscle and therefore [Mb] are present in the forehead regions (Ferrari, Mottola & Quaresima, 2004; Scheeren, Schober & Schwarte, 2012). Total haemoglobin [tHb] is determined by adding the concentration changes of [O<sub>2</sub>Hb] and [HHb] and as haematocrit is assumed constant, [tHb] reflects the cellular blood volume, which has been shown to alter significantly in response to brain activation (Buxton, Wong & Frank, 1998; Ehlis et al., 2005; Trampel & Turner, 2012; Plichta et al., 2007b; Tamura, Hoshi & Okada, 1997). The total haemodynamic response can be characterised as the collective changes in blood flow, blood volume and oxygenation that accompany a physiological or cognitive stimulus (Hu, Hong & Ge, 2013).

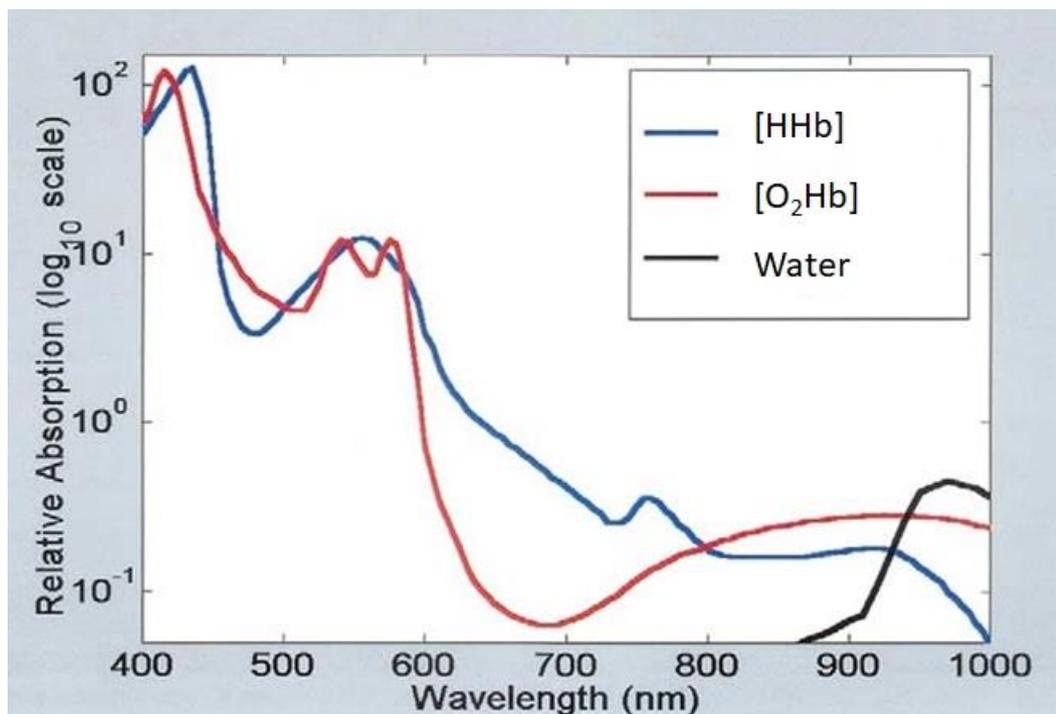


Figure 4: Absorption spectra of [O<sub>2</sub>Hb], [HHb] and water. Note the distinctive cross-over of the dominant chromophore occurs at ~800 nm. (image adapted from Strangman, Boas & Sutton, 2002, p. 680)

### 2.5.1 Operational parameters

Because of the relative transparency of biological tissue, near infrared red light is able to propagate several centimetres into the tissue (Boas et al., 2014). Light is emitted from a source on the NIRS device and attenuation of returning light is used to determine the properties of the tissue below (Boas et al., 2014). NIRS recordings are usually made with the source (emitter) and detector on the same side of the head, and this produces what is known as a diffuse trajectory (Pellicer and del Carmen Bravo, 2011). The optical pathlength through biological tissue is longer than the physical distance between emitter and detector due to the scattering effect (Hoshi, 2003) causing light to pass through the tissue along an elliptical (“banana shaped”) path though the tissue from emitter to detector, with the largest cross-section of the sample located in the middle (Ehlis et al., 2005; Gratton, Maier, Fabiani, Mantulinm & Gratton, 1994) (see Figure 5). The curvilinear nature of the light path results from both the scattering properties of the tissue and the photon diffusion path (Ekkekakis, 2009; Ferrari & Quaresima, 2012). In order to form this characteristic curvilinear path scattering of light must be higher than absorption, lower values would result either in the light travelling in a straight line or in the absorption of all light passing through the tissue (Ekkekakis, 2009). In relation to proton diffusion, although protons may follow an infinite number of paths some are more likely than others and the ‘likely’ path has a higher density in a crescent shape as shown in Figure 5 (Gratton et al., 1994; Ekkekakis, 2009).

The detector is usually positioned 2-7cm away from the emitter (Villringer & Chance, 1997) with the depth of penetration being directly proportional to the distance between emitter and detector (Ekkekakis, 2009; Ferrari & Quaresima, 2012; Scheeren, Schoder & Schwarte, 2012). The penetration depth of the signal is approximately half the interoptode distance and therefore a minimum distance of 2.5 cm is recommended in order to ensure that the tissue sampled does not just contain extracerebral tissue (Pellicier & del Carmen Bravo, 2011; Van der Zee, Arrige, Cope & Delpy, 1990). The assumption that differences in light attenuation arise from alterations in concentrations of  $[O_2Hb]$  and  $[HHb]$  is used to in order to quantify values of individual chromophores using different wavelengths of infrared light (Delpy & Cope, 1997; Haeussinger, Heinzl, Hahn, Schecklmann, Ehlis & Fallgatter, 2011; Kohl et al., 1998). Light entering the tissue can undergo two possible interactions; absorption or scattering (Villringer & Chance, 1997) and a combination of these is responsible for the light attenuation that occurs when near infrared light passes through the tissue (Villringer & Chance, 1997). In the NIR range it is

expected that 80% of light lost is due to scattering and 20% is lost due to absorption (Pellicer & del Carmne Bravo, 2011).

In order to quantify the changes in chromophore concentrations the factor by which scattering increases optical path length (differential path length factor (DPF)) must be known (Delpy & Cope, 1997; Ekkekakis, 2009; Perrey, 2008). In order to account for the loss of photons resulting from the scattering of light a correction factor  $G$  is introduced to represent the cosine of the scattering angle (Ekkekakis, 2009). Experimental measurements of a range of tissues have demonstrated that the DPF is a relatively constant function of optode spacing and therefore can be quantified, although movement of optodes during measurement may contribute to changes in the DPF causing errors in the measurement (Essenpreis, Elwell, Cope, Van der Zee, Arridge & Delpy, 1993; Hoshi, 2011; Perrey, 2008; Porcelli, Marzorati, Lanfranconi, Vago, Pišot & Grassi, 2010). In addition to assessing chromophore concentrations, cerebral blood flow (CBF) and cerebral blood volume (CBV) can also be detected using NIRS (Pellicer & del Carmen Bravo, 2011). An increase in cerebral blood flow occurs in response to increased neuronal activation and is usually due to increased blood flow velocity within the capillaries (Villringer & Chance, 1997).

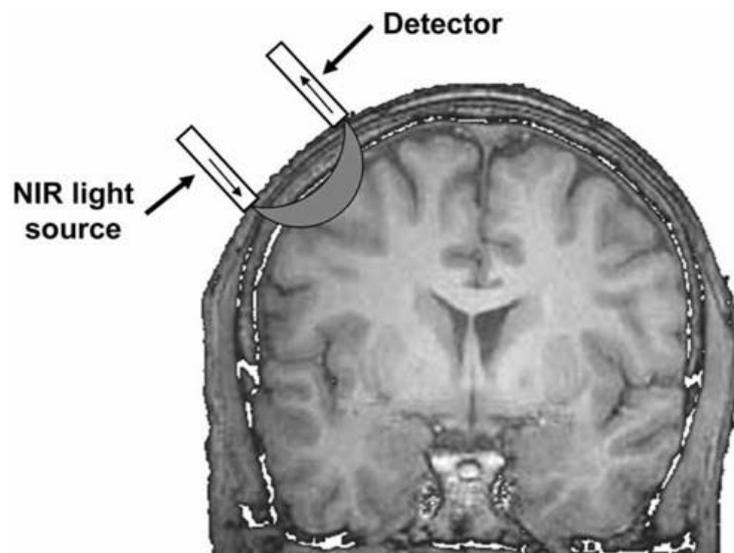


Figure 5: The curvilinear path of NIR light through the tissue from emitter to detector (image from Ekkekakis, 2009, p. 510).

### 2.5.2 History and development of NIRS

The first application of NIRS to monitor *in vivo* changes of brain oxygenation was described by Jobsis (1977) who used the technology to monitor changes in an intact cat head. The use of NIRS for assessment of cognitive function in humans initiated with the development of a single channel system in 1989 (Ferrari & Quaresima, 2012). Although now commonly used to assess changes in muscle oxygenation the use of NIRS began as a technique to monitor changes in cerebral oxygenation (Wolf, Ferrari & Quaresima, 2007). Many multiple position NIRS systems (fNIRS) are now available and are growing in popularity for cognitive research with over 250 publications per year currently using fNIRS devices (Yücel et al., 2017), however, until the early 21<sup>st</sup> century single position systems were the only ones available on the market (Ehlis et al., 2005). These single position systems are still commercially available and their low cost and therefore high accessibility means that they are still being used for cognitive research despite the fact that fNIRS systems provide more detailed information (Cheung et al., 2014; Debevec & Mekjavic, 2012; Oussaidene, Prieur, Bougault, Borel, Matran & Mucci, 2013; Rupp et al., 2013).

### 2.5.3 Types of NIRS devices

There are three specific types of NIRS devices: continuous wave (cw-NIRS), frequency domain (FD-NIRS), and time domain (TD-NIRS) (Ferrari & Quaresima, 2012). The most commonly used NIRS devices are continuous wave (cw-NIRS) devices (Boas et al., 2014). These devices rely on a constant stream of infrared light which is monitored by a detector positioned a fixed distance from the light emitter (Perrey, 2008) and simply measure attenuation of NIR light returning from the tissue (Ekkekakis, 2009; Ferrari & Quaresima, 2012; Scholkmann et al., 2014; Tak & Ye, 2014). Relative changes in oxygenation variables are determined and presented in arbitrary units as the assumptions of tissue homogeneity do not allow for absolute determinations of [O<sub>2</sub>Hb] and [HHb] (Hoshi, 2003; Obrig & Villringer, 2003; Scholkmann et al., 2014; Toronov et al., 2001), although a study by Stone, Fryer, Ryan & Stoner (2006) has demonstrated that absolute [tHb] can be accurately determined using cw-NIRS. These devices rely on calculations derived using the modified Beer-Lambert law (MBLL) (see section 2.5.4 for more detail) and are the most readily available commercial instruments (Hoshi, 2007).

Whilst multi-position functional near infrared spectroscopy (fNIRS) devices are the most commonly used devices for cognitive research using NIR technology, single position,

fixed distance devices are able to accurately measure changes in  $[O_2Hb]$  and  $[HHb]$  (Ferrari, Mottola & Quaresima, 2004). FD-NIRS devices allow determinations to be made regarding both scattering properties as well as absorption changes and therefore are able to measure both attenuation and phase delay of returning light (Ferrari & Quaresima, 2012; Scholkmann et al., 2014; Tak & Ye, 2014; Toronov et al., 2001). TD-NIRS utilises short pulses of light in order to detect the shape of the pulse after passage through the tissue allowing the temporal characteristics of the haemodynamic response to be investigated (Ferrari & Quaresima, 2012; Scholkmann et al., 2014; Tak & Ye, 2014).

The Artinis Portalite NIRS system investigated in this thesis is a cw-NIRS device. It weighs 84g and is particularly compact measuring 83 x 52 x 20mm. It emits infrared light at the wavelengths of 760 and 850 nm and has three LEDs with a source-detector distance of 30, 35 and 40mm allowing for spatially resolved spectroscopy (SRS) measurements to be undertaken (McManus, Collision & Cooper, 2018). SRS uses the light sources placed at different distances from the detector to make a distinction between light that has travelled a shorter distance (less depth penetration) and light that has travelled a longer distance (greater depth penetration) (Gagnon et al., 2002). During data acquisition Bluetooth technology is used to connect the device to a laptop computer and the Artinis Oxymon ([www.artinis.com](http://www.artinis.com)) software displays the data and can subsequently be used for data analysis.

#### **2.5.4 The Beer-Lambert law**

The Beer-Lambert law is used to describe the attenuation of light measured in optical density in relation to the property of the material through which it travels (Pellicier and del Carmen Bravo, 2011). This law states that when light passes through a coloured compound some of the light will be absorbed and the reduction in intensity (attenuation) of the emerging light can be used to determine the properties of the compound (Perrey, 2008). The Beer-Lambert law cannot be directly used to examine the absorption rate of light passing through biological tissue as it is not valid in a medium where scattering can occur (Scholkmann et al., 2014). Consequently, a modified Beer-Lambert law (MBLL) developed by Delpy, Cope and Van der Zee (1988) which takes into account the scattering of light, is used in NIRS measurements.

The MBLL on which the measurements from most cw-NIRS systems are based makes three main assumptions 1) Scatter is high but changes negligibly throughout the

measurement; 2) the medium in which changes are monitored is homogenous; and 3) changes in the volume sampled are homogenous (Ferrari & Quaresima, 2012; Obrig and Villringer, 2003). The assumption regarding scatter is acceptable because absorption is more significantly affected by changes in CBF than scatter (Obrig & Villringer, 2003; Ekkekakis, 2009). The second and third assumptions, however, do present some issues as no biological tissue can support the assumption of homogeneity (Delpy & Cope, 1997; Obrig & Villringer, 2003). This is a particular problem in the brain as changes in activation are regional, and therefore do not occur homogeneously throughout the tissue (Ekkekakis, 2009). The MBLL requires light lost due to other attenuation factors to be known and consequently this equation cannot be used to quantify an absolute calculation of chromophore concentration (Obrig & Villringer, 2003; Pellicer & del Carmen Bravo, 2011).

### **2.5.5 Advantages of NIRS**

One of the greatest advantages of the use of near infrared spectroscopy is the portability, flexibility and non-invasive nature of the method (Perrey, 2008; Scholkmann et al., 2014; Schroeter, Zysset, Kupka, Kruggel & Von Cramon, 2002; van Beekvelt, Van Engelen, Wevers & Colier, 2002). The relatively straightforward nature of the technology also means that it can be operated with minimal training (Perrey, 2008). It allows continuous measurements of haemodynamic changes within the tissue and has a high temporal resolution (0.5-1s) allowing assessments of cerebral blood flow and changes in chromophore concentrations to be made in 'real time' (Hoshi, 2003; Hoshi, 2007; Hoshi, 2011; Kakimoto, Nishimura, Hara, Okada, Tanii & Okazaki, 2009; Plichta et al., 2007a; Van Beekvelt, Colier, Wevers & Van Engelen, 2001). NIRS is also capable of biochemical specificity as the chromophores [O<sub>2</sub>Hb] and [HHb] absorb light at a known point of the infrared spectrum (Ferrari & Quaresima, 2012; Perrey, 2008; Schroeter, Zysset & Cramon, 2003; Villringer & Chance, 1997). The non-invasive and safe nature of the technology also means that there is no limit to the number of repeated measures that can be undertaken (Hoshi, 2007; Perrey, 2008; Scheeren, Schober & Schwarte, 2012; Strangman, Boas & Sutton, 2002).

Whilst NIRS measurements can be affected by movement artefacts, it is much more robust to these artefacts than techniques such as EEG and there is generally no restriction required to movement (Anderson et al., 2018; Derosière, Mandrick, Dray, Ward & Perrey, 2013; Ferrari et al., 2014; Tak & Ye, 2014) meaning a larger number of research questions can be answered with this technique than with any other imaging modality (Derosière et al., 2013; Strangman et al., 2006). The lack of restriction of subject movement increases

relaxation, reduces anxiety and therefore has minimal influence on the completion of cognitive tasks (Anderson et al., 2018; Tamura, Hoshi & Okada, 1997; Yanagisawa et al., 2010). Furthermore, it promotes ecological validity of experimental conditions as subjects can be examined during movement or in a natural environment, which is crucial in psychological research (Ferrari et al., 2014; Hoshi, 2007; Hoshi, 2011; Kakimoto et al., 2009; Villringer & Chance, 1997), including the ability to take measurements during exercise (Tempest, Eston & Parfitt, 2014). CW-devices can be small, lightweight and wireless making them wearable and completely unobtrusive (Perrey, 2008; Scholkmann et al., 2014; Yanagisawa et al., 2010; Yücel et al., 2017). The portability of the NIRS devices also means that the technique lends itself well to the study of population changes over an extended period of time (Perrey, 2008; Vinette et al., 2015).

NIRS is a valuable tool for research particularly in situations where other neuroimaging techniques are difficult to utilise (Plichta et al., 2007a,b), and it is suitable for use with children and clinical populations in 'real world' settings where other methods might not be appropriate or practical (Bendall, Eachus & Thompson, 2016; Ferrari et al., 2014; Mckendrick et al., 2014; Yücel et al., 2017). In addition, NIRS devices produce no noise disturbance, unlike functional magnetic resonance imaging (fMRI) or (positron emission tomography (PET) scanners (Ferrari et al., 2014; Plichta et al., 2007a,b). NIRS is also relatively inexpensive and simple to use when compared to other brain imaging techniques (Cui, Bray, Bryant, Glover & Reiss, 2011; Plichta et al., 2007b; Scroeter, Zysset & Crannon, 2003; Yücel et al., 2017) and the simplicity of the set-up means experimental set-up time is substantially reduced (Bendall, Eachus & Thompson, 2016). Near infrared light does not interfere with electromagnetic radiations meaning that measurements can be obtained simultaneously with fMRI and PET measurements (Bendall, Eachus & Thompson, 2016; Hoshi, 2011; Steinbrink, Villringer, Kempf, Haux, Boden & Obrig, 2006; Tamura, Hoshi & Okada, 1997). Furthermore, when combined with EEG, investigations of electrical and haemodynamic changes can be made simultaneously (Bendall, Eachus & Thompson, 2016). Because NIRS examines the optical properties of tissue rather than the radioactive (as with PET) or magnetic (as with fMRI) the application of a contrast medium or need for extensive technical arrangements are eliminated (Obrig & Villringer, 2003; Plichta et al., 2007a,b).

NIRS also has advantages over blood oxygen level dependent (BOLD) fMRI as this technique only allows the quantification of [HHb] whereas NIRS allows for the quantification of [O<sub>2</sub>Hb] and [tHb] in addition to [HHb] and therefore can provide more detailed information regarding the cerebral metabolic rate of oxygen (CMRO<sub>2</sub>) (Perrey,

2008). Given that neurological activation measured by NIRS devices can be characterised by increases in [O<sub>2</sub>Hb] and [tHb] without detectable changes in [HHb] (Hoshi & Tamura, 1993; Kato et al., 1993; Kleinschmidt et al., 1996), the NIRS devices may be capable of detecting changes too small to be shown in a BOLD fMRI study (Obrig & Villringer, 2003). In fact a study by Mehagnoul-Schipper and colleagues (2002) demonstrated that task related decreases in [HHb] were observed by both NIRS and fMRI in response to increased cognitive demands, and simultaneous increases in [O<sub>2</sub>Hb] were observed by the NIRS for both younger and elderly participants, a result which would not have been detected by BOLD fMRI alone.

### **2.5.6 Limitations of NIRS**

One limitation of NIRS data quantification is related to the attenuation of light. In biological tissue attenuation of light comes from light scattering as well as light absorption (Delpy & Cope, 1997; Obrig & Villringer, 2003). A non-linear relationship is created between light absorption and attenuation, and the absorption coefficient ( $\mu_a$ ) of the majority of tissues is smaller than the scattering coefficient ( $\mu_s$ ) (Cheong, Prah & Welch, 1990; Delpy & Cope, 1997). Diffusion theory, which models scattering and absorption of light, makes the assumption that tissues are homogenous (Delpy & Cope, 1997; Toronov et al., 2001). This presents issues when interrogating biological tissue as there are no biological tissues which can be examined non-invasively without the light passing through an intervening layer (Delpy & Cope, 1997). For example, when investigating neural activity the light must pass through the scalp, skull and cerebrospinal fluid (CSF) before penetrating the cerebral cortex (Delpy & Cope, 1997; Fukui, Ajichi & Okada, 2003; Perry, 2008), although the CSF layer has low scattering properties which may actually be advantageous to the sensitivity of the NIRS signal to absorption changes (Okada & Delpy, 2003). Moreover, the assumptions with regards to scattering and photon diffusion made by the MBL (see section 4.14) are also potential sources of error within the NIRS measurement (Ekkekakis, 2009).

Individual subject characteristics can influence the quality of the NIRS signal. Melanin on the surface of the skin also absorbs NIR light and can therefore contribute to the attenuation observed (Canning & Scheutz, 2013; Ferrari, Mottola & Quaresima, 2004; Scheeren, Schober & Schwarte, 2012). Race is therefore one of a number of demographic cofounders which must be considered when comparing groups with NIRS measurements, the others being; age (cortical responses are less pronounced in older subjects), gender and handedness (Ferrari & Quaresima, 2012; Orihuela-Espina, Leff, James, Darzi & Yang, 2010).

Wherever possible groups should be carefully matched to minimise the effect of these confounds.

Light scattering can also be influenced by activities occurring at neuronal membranes (Villringer & Chance, 1997). Although light must pass through several non-cerebral tissues, these have a smaller blood content than cerebral tissue meaning that NIRS can still be considered suitable for interrogating cerebral activation (Villringer & Chance, 1997). There is likely, however, to be some level of attenuation of light due to extracerebral tissues and information from cerebral activation may be contaminated by haemodynamic changes in the extracerebral microvasculature (Firbank, Okada & Delpy, 1998; Hoshi, 2011; Selb et al., 2014; Tak & Ye, 2014; Young, German, Barnett, Manara & Nelson, 2000). This involvement of extracellular tissue has been cited as the leading limitation in the use of NIRS for assessing cognitive function as changes in forehead skin perfusion may cause an overestimation of cortical activation or alternatively obscure signals arising from brain activation (Boas et al., 2014; Ferrari et al., 2014; Hoshi, 2011; Yücel et al., 2017). In fact, a study by Haeussinger et al. (2011) indicated that as little as 3% of light attenuation may be due to absorption by cerebral tissue. The issue of selective quantification of signals arising from cerebral tissue rather than extracerebral tissue is the biggest barrier to the acceptance of NIRS in cognitive research (Ferrari, Mottola & Quaresima, 2004; Hoshi, 2007; Scheeren, Schrober & Schwarte, 2012). When using NIRS to examine brain activity you must be able to assume that there is no distinct change in extracerebral blood flow or oxygenation before changes in chromophore concentrations observed can be attributed to alterations in brain activity (Villringer & Chance, 1997). This assumption must also take into account the spontaneous fluctuations to skin and cerebral blood flow that occur naturally during the resting state, including haemodynamic changes due to systemic vascular responses from outside of the brain (Hoshi, 2011; Perrey, 2008). Furthermore, NIRS is sensitive to global changes in blood flow as well as local regional changes which may contribute to the measurements obtained (Toichi et al, 2004).

NIRS also lacks spatial resolution and depth penetration (limited to ~ 30mm) meaning that studies using this technique are limited to interrogating surface cortical structures and activation in subcortical regions and deep brain structures cannot be measured (Bendall, Eachus & Thompson, 2016; Hoshi, 2003; Plichta et al., 2007a; Tak & Ye, 2014; Tempest, Eston & Parfitt, 2014; Villringer & Chance, 1997). In addition to the depth penetration of ~30mm, regional interrogation on either side that can be measured (perpendicular to the source-detector axis) is limited to ~10mm (Canning & Scheutz, 2013).

The accuracy of this depth measurement is of course dependent on the thickness of the skull and CSF, and the thickness of the CSF layer can vary with movement, postural changes and small expansions of the brain (Custo, Wells, Barnett, Hillman & Boas, 2006; Okada & Delpy, 2003; Ferrari, Mottola & Quaresima, 2004).

Due to the inability to accurately determine the pathlength of the optical signal absolute concentration changes are also not available in the majority of NIRS devices (including the Artinis Portalite which is examined in this thesis), thus it is only possible to examine relative concentration changes in the chromophores of interest (Hoshi, 2007; Hoshi, 2011; Simonson & Piantadosi, 1996; Villringer & Chance, 1997). However, that does not eliminate the usefulness of NIRS devices in cognitive research as in the majority of cases it is more important to determine a change in brain activity relative to another condition or time point rather than to quantify that change in absolute terms (Scholkmann et al., 2014).

NIRS lacks anatomical brain information, therefore NIRS optodes are generally positioned using the 10-20 or 10-10 EEG positioning system (Jurak, Tsuzuki & Dan, 2007; Mehagnoul-Schipper et al., 2002; Tak & Ye, 2014; Yücel et al., 2017) and this technique for positioning of the probes may not be exact, leading to a potential false signal change when comparing a single subject across multiple sessions or errors in comparing cortical activation between groups (Hoshi, 2011; Orihuela-Espina et al., 2010; Plichta et al., 2007a,b). In addition to limitations in comparing a single subject across multiple sessions there are issues which need to be considered when attempting to make a between-groups comparison. Differences in head size and shape as well as differences in skull thickness and anatomical brain structures vary between participants but the emitter-detector distance is fixed meaning that inter-subject variation in the brain region interrogated is unavoidable (Gratton et al., 1994; Hoshi, 2011; Okada & Delpy, 2003).

A further issue with NIRS measurements arises from the coupling of the sources and detectors to the head, as every movement can cause a decoupling of the optode from the head which may cause a change in the intensity of light detected or be reflected as a motion artefact in the measured signal (Brigadoi et al., 2014; Orihuela-Espina, et al., 2010; Tak & Ye, 2014). These motion artefacts can mask the true haemodynamic response and thus it is necessary to remove them from the data. This can be done by using an additional sensor to detect movement, by eliminating all trials with motion artefacts or by using some form of post-processing of the data such as applying a Gaussian filter (Brigadoi et al., 2014; Molavi & Dumont, 2012; Tak & Ye, 2014; Wang, Wu, Mao, Fu & Hsu, 2010). As the motion

artefacts are typically characterised by rapid changes resulting in sharp spikes in the data signalling processing methods can be used to remove them (Cui, Bray & Reiss, 2009; Tak & Ye, 2014; Yücel et al., 2017). The decoupling of the probe from the head can be minimised, particularly within the frontal regions by using bi-adhesive tape to securely affix the plastic casing surrounding the optode to the subjects' forehead (Scheeren, Schober & Schwarte, 2012). It must be noted that movement artefacts are not only caused by decoupling of the probes from the head but also by head movements and orientation, jaw movements and facial expressions (Brigadoi et al., 2014; Canning & Scheutz, 2013; Cui, Bray & Reiss, 2009; Perrey, 2008). Despite the effect of motion artefacts on the data the NIRS signal is still more robust against movement than any of the other imaging techniques. A minor issue with NIRS measurements is insufficient shielding of the optode permitting ambient light to reach the detectors (Orihuela-Espina et al., 2010), however, this issue is easily overcome by wrapping the area in bandage or covering the optode with dark opaque material (Hoshi, Shimada, Sata & Iguchi, 2005; Meek et al., 1995; Obrig et al., 2002; Wyatt et al., 1990). This covering can also be used to assist with holding the sensors in place (Canning & Scheutz, 2013).

In cognitive NIRS studies  $[O_2Hb]$  is most commonly used to assess changes as it generally shows the highest correlations with task performance than  $[HHb]$  (Hoshi, Kobayashi & Tamura, 2001), however, there are some indications that  $[O_2Hb]$  is more sensitive to extracerebral contaminations such as changes in heart rate and blood pressure (Boden, Obrig, Kohncke, Benav, Koch & Steinbrink, 2008). One particular limitation of single position NIRS devices is that it is often difficult to determine the correct place to position to NIRS probe as brain activity in response to a stimulus is highly location specific (Scholkmann et al., 2014). Moreover, compared to EEG which registers signals evoked milliseconds after the stimulus is presented the haemodynamic response measured by NIRS and fMRI is relatively slow (Scholkmann et al., 2014), meaning that time delay of the NIRS signal is also an issue in short duration tasks. The response initiates approximately 2 seconds after the presentation of the stimulus and peaks at around 4-8 seconds after the stimulus onset (Canning & Scheutz, 2013; Leff et al., 2011; Straight & Scheutz, 2014) taking 10-12 seconds to return to baseline as homeostasis is re-established (Boynton, Engel, Glover & Heeger, 1996; Buckner et al., 1996; Canning & Scheutz, 2013) (see Figure 6). Consequently, if stimuli are presented in close succession a temporal overlap of the haemodynamic response may be observed (Plichta et al., 2007a) and an artificially elevated haemodynamic response to a cognitive challenge may be recorded (Liu et al., 2004).

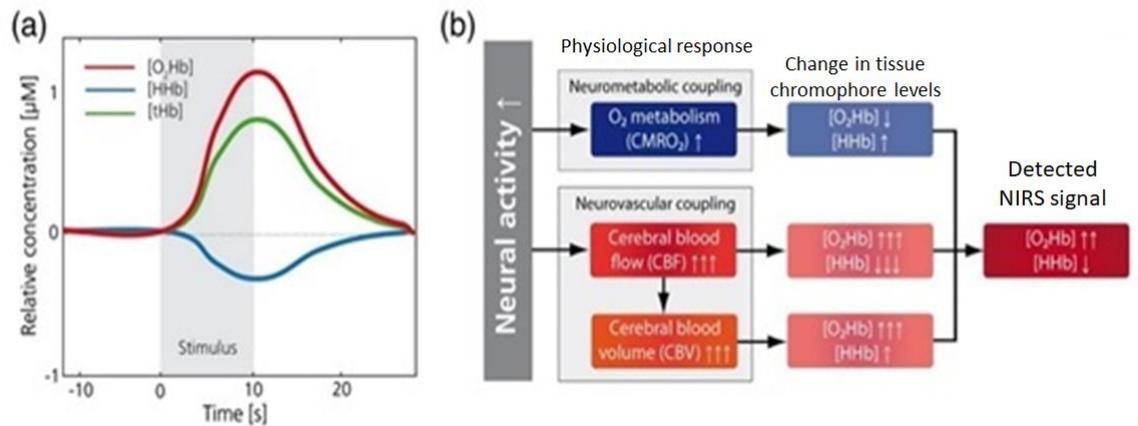


Figure 6: (a) Time evoked changes in haemodynamic response to increased neural activity and (b) central haemodynamic changes and their effect on NIRS signals (image adapted from Scholkmann et al., 2014, p. 17).

The above factors are commonly recognised as limitations for the use of NIRS measurements and whilst most can be overcome with careful experimental control and do not override the advantages of the NIRS they must all be considered when interpreting any results obtained using cw-NIRS devices.

### 2.5.7 Assessment of cognitive function

In the near infrared range light is easily able to pass through the scalp and skull (Boas et al., 2014; Ehlis et al., 2005) and NIRS uses this NIR light to assess alterations in cerebral perfusion (Scheckleemann, Ehlis, Plichta & Fallgatter, 2008). The frontal region is the easiest to investigate as there is a lack of signal contamination from hair (Cui et al., 2011; Dersosière et al., 2013; Lloyd-Fox, Blasi & Elwell, 2010; Yücel et al., 2017). The two dominant chromophores in the NIR spectrum, [O<sub>2</sub>Hb] and [HHb] are also the most relevant for identifying changes in neurological activation (Strangman, Boas & Sutton, 2002). A portion of the light emitted from the NIRS device has passed through cerebral tissue and therefore changes in cerebral haemoglobin concentration can be revealed (Boas et al., 2014). Nerve cells have an elevated metabolic rate and consequently an increase in neuronal activation leads to increased blood flow in the activated region due to metabolically and neuronally transmitted vasodilation (Duschek, Heiss, Schmidt, Werner & Schuepbach, 2010; Iadecola, 2004). An increase in cognitive demands therefore leads to augmented cerebral blood flow (Logothetis, Pauls, Augath, Trinath & Oeltermann, 2001).

NIRS relies on the tight coupling between neural activation and the cerebral haemodynamic response, described as neurovascular coupling (Duschek et al., 2010; Scholkmann et al., 2014; Villringer & Chance, 1997; Yücel et al., 2017) and therefore changes in the haemodynamic response can be used to infer neural activity (Anderson et al., 2018; Brigadoi et al., 2014). Modulations in blood flow during cognition reflect a constant adjustment to fluctuating metabolic demands, particularly for [O<sub>2</sub>Hb] and glucose (Duschek et al., 2010; Paulson, 2002), and increased neural activity causes an increased metabolic demand in the brain leading to an increase in consumption of both (Ferrari, Bigand, Perrey & Bugajska, 2014; Scholkmann et al., 2014; Villringer & Chance, 1997). While oxygenation, rCBF and glucose have the same directional increase in response to neural activation, rapid vasodilation overshoots the need for oxygen meaning that the increases in oxygenation are disproportionate (Paulson, Hasselbalch, Rostrup, Knudson & Pelligrino, 2010; Perrey, 2008). An increase in cerebral blood flow would therefore be expected to lead to an improvement in functional conditions within the region which in turn would support enhanced cognitive processing (Duschek & Schandry, 2004). Conversely, the reverse relationship could be true, in that an increase in cerebral blood flow is the result of increased neural activation meaning that the increased activation rather than the increased blood flow would be responsible for enhanced cognitive function (Duschek et al., 2010).

Changes in [O<sub>2</sub>Hb] and [HHb] reflect alterations in the cerebral metabolic rate (CMRO<sub>2</sub>) whereas [tHb] reflects changes in cerebral blood flow (CBF) (Gagnon et al., 2012; Perrey, 2008; Tamura, Hoshi & Okada, 1997). An increase in neural activity is expected to be characterised by increases in [tHb] and [O<sub>2</sub>Hb] coupled with a decrease in [HHb] (Leff et al., 2011; Plichta et al., 2006; Perrey, 2008; Schecklmann, Ehlis, Plichta & Fallgatter, 2008). Although there is a lack of consensus as to which chromophores give the best indication of neural activation and it is therefore important that all three are reported to give a complete picture of findings (Ehlis et al., 2005; Ekkekakis, 2009; Plichta et al., 2006). One issue in imaging studies is the intrinsic fluctuations in brain activity that occur during the resting state and which must be considered when making comparisons to the activated state (Cordes et al., 2001; Cordes, Haughton, Carew, Arfanakis & Maravilla, 2002; Tamura, Hoshi & Okada, 1997). These fluctuations are often due to physiological noise such as respiration and arterial pulse oscillations and blood pressure Mayer waves (Boas et al., 2004; Canning & Scheutz, 2013; Hoshi, 2003; Hu, Hong & Ge, 2013). The fluctuations can at times mimic those evoked by changes in functional activity (Hoshi, 2003) and may even be a result of interactions between distant brain regions (Hu, Hong & Ge, 2013). Furthermore, both scalp

blood flow and blood flow to the cerebral cortex fluctuate spontaneously during resting conditions (Hoshi, 2011; Hoshi & Tamura, 1997; Toronov et al., 2000) which must be considered when assessing the haemodynamic response relative to a resting baseline.

A range of different changes can occur in response to increased neurological activation. An increase in [tHb] and [O<sub>2</sub>Hb] with an associated decrease in [HHb] may be observed (Causse, Chua, Peysakhovich, Del Campo & Matton, 2017; Hauessinger et al., 2011; Obrig & Villringer, 2003), however, an increase in [tHb] and an increase in [O<sub>2</sub>Hb] may also be observed without a decrease in [HHb] (De Joux et al., 2017; Hoshi & Tamura, 1993; Kato et al., 1993; Kleinschmidt et al., 1996). Or even an early deoxygenation leading to an increase in [HHb] (Buxton, Wong & Frank, 1998). In the same way that activation is usually characterised by an increase in [tHb] and [O<sub>2</sub>Hb] with an associated decrease in [HHb], deactivation is characterised by the reverse, a decrease in [tHb] and [O<sub>2</sub>Hb] coupled with an increase in [HHb] (Hoshi & Tamura, 1997). Deactivation may also be identified by a decrease in rCBF (Obrig & Villringer, 2003). Spatially resolved spectroscopy (SRS) is used to determine the tissue saturation index [TSI] or oxygen saturation which can be derived using proton diffusion theory to measure the optical density change as a function of multiple distances and represents the ratio of [O<sub>2</sub>Hb] to [HHb] (Delpy & Cope, 1997; Ferrari, Mottola & Quaresima, 2004; McManus, Collision & Cooper, 2018; Saito et al., 2008).

In addition to [O<sub>2</sub>Hb] which can be used to indicate an increase in oxygen delivery, the difference between [O<sub>2</sub>Hb] and [HHb] which is known as [Hb<sub>diff</sub>] and calculated using the equation;  $[Hb_{diff}] = ([O_2Hb] - [HHb])/2$  can be used to indicate oxygen utilisation (Claassen, Colier & Jansen, 2006; Tempest, Eston & Parfitt, 2014; Van Beekvelt et al., 2001; Yoshitani et al., 2007). As oxygen metabolism is efficient (in other words all oxygen dissociated from haemoglobin is likely to be metabolised), this value reflects the difference between oxygen delivery and oxygen consumption (Bhambhani et al., 2006; Ekkekakis, 2009). An increase in [tHb] and an increase in [O<sub>2</sub>Hb] without an increase in [Hb<sub>diff</sub>] is likely to indicate that increases in cerebral blood supply exceeded demands induced by increased neural activity (Hoshi & Tamura, 1993). While [Hb<sub>diff</sub>] and [TSI] values are similar, they do represent different aspects of the haemodynamic response and therefore should both be reported.

Changes in [tHb] can be used to indicate changes in blood flow within the region of interest (Hoshi & Tamura, 1993; Van Beekvelt et al., 2001) although some authors have determined [O<sub>2</sub>Hb] to be the most sensitive indicator of rCBF changes (De Joux et al., 2017;

Hoshi, 2003). The oxygen supply and utilisation relationship provides information on localised brain activity that could not be determined by assessing changes in CBF alone (Hoshi & Tamura, 1993). Changes in [HHb] are used in BOLD fMRI studies to indicate responses to cognitive tasks, however, in NIRS studies increases in [O<sub>2</sub>Hb] often prove more robust indicators of regional activation (Hoshi, Kobayashi & Tamura, 2001; Hoshi & Tamura, 1993). In addition [O<sub>2</sub>Hb] measures have higher spatial correlations than [HHb] measures (Hofmann et al., 2008; Plichta et al., 2007a).

During cortical activation, a neurovascular response is initiated causing dynamic changes in rCBF, CBV and the rate of oxygen consumption (CRMO<sub>2</sub>) which can be monitored in real time by measuring changes in haemoglobin levels (Perry, 2008). This often manifests in an increase in cerebral blood flow exceeding the consumption of oxygen [O<sub>2</sub>] and leads to an increase in intravascular levels of [O<sub>2</sub>Hb] (Perrey, 2008; Villringer & Chance, 1997). Increases in neural activity are usually characterised by an initial decrease in [O<sub>2</sub>Hb] (increase in [HHb]) followed by an extended increase in [O<sub>2</sub>Hb] (Leff et al., 2011; Plichta et al., 2007b; Villringer & Chance, 1997), this initial decrease in [O<sub>2</sub>Hb] is described as the *inverse response phase* and is likely to reflect increased utilisation prior to an increase in oxygen delivery (Leff et al., 2011). The initial phases of the response may also be characterised by an initial decrease in [HHb] accompanied by an increase in [O<sub>2</sub>Hb] (Perrey, 2008; Plichta et al., 2007).

Haemodynamic response is usually assessed by comparing mean changes in the vascular response to baseline readings, or alternatively by correlating mean values to behavioural data (Schroeter et al., 2002). However, it must be considered that just because a haemodynamic response correlates with a behavioural response, this does not mean that the increased cognitive load is responsible for the altered haemodynamic response as it could be caused by co-occurring neural activity (Canning & Scheutz, 2013). Extensive research has detailed the haemodynamic response associated with brain activity, yet little knowledge exists about the response following deactivation of a particular brain region (Villringer & Chance, 1997), it is likely, however, to be characterised by a decrease in both [O<sub>2</sub>Hb] and [HHb] (Obrig & Villringer, 2003). As the majority of NIRS devices do not enable absolute concentrations of [O<sub>2</sub>Hb] and [HHb] relative changes are quantified using arbitrary units (A.U.) (Toronov et al., 2001).

Conditions requiring sustained attention to a target or in response to a higher level of task complexity have been shown to increase [O<sub>2</sub>Hb] levels above that generally

observed in response to other cognitive demands (Ayaz, Shewokis, Bunce, Izzetoglu, Willems & Onaral, 2012; Causse et al., 2017; Derosi re et al., 2013; Kojima & Suzuki, 2010) indicating that the [O<sub>2</sub>Hb] response is task specific. Regional brain activation is accompanied by an increase in rCBF (Ehlis et al., 2005; Ekkekakis, 2009) and changes in regional CBF (rCBF) are observed in the same direction as changes in [O<sub>2</sub>Hb] and are by themselves indicative of underlying neural activity (Buxton, Wong & Frank, 1998; Hoshi, 2007). Small changes in rCBF are not always reflected by changes in [tHb], and therefore [O<sub>2</sub>Hb] may be more sensitive to rCBF changes (Buxton, Wong & Frank, 1998; Hoshi, 2007). When attempting to map cerebral response rather than examine localised regional changes, however, [tHb] may provide a better indicator (Gaganon et al., 2012), particularly as the values for [tHb] provide an overview of blood flow changes.

In addition to assessing changes related to the presentation of a cognitive stimulus in a single session, NIRS can be used to assess learning. Following learning a decreased magnitude of activity resulting from a reduced time of processing (more automatic processing) and a lower effort requirement is likely to be observed (Ikegami & Taga, 2008; McKendrick et al., 2013; Poldrack, 2000), this reduced neural activity would be reflected in the haemodynamic response with a mediation of task related increases in [O<sub>2</sub>Hb] and changes in [HHb] (Hatakenaka, Miyai, Mihara, Sakoda & Kubota, 2007; Ikegami & Taga, 2008; Matsui, Tanaka, Yonezawa & Kurachi, 2007). This effect is particularly apparent in the prefrontal cortex (McKendrick et al., 2014; Poldrack, 2000) and the haemodynamic response appears to alter relative to the amount of time spent on skill training (McKendrick et al., 2014).

### **2.5.8 Validity of NIRS for assessing neurological responses**

The [HHb] concentration measured by NIRS is proportional to the blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) and there is a close agreement between these two methods (Buxton, Wong & Frank, 1998; Huppert, et al., 2006; Toronov et al., 2001), including a temporal correlation between the measurements obtained (Toronov et al., 2001). Some studies have also shown a correlation between blood volume changes detected by NIRS and BOLD fMRI (Kleinschmidt et al., 2006). The best correlation is usually observed between [HHb] changes and the BOLD signal (Alderliesten et al., 2014; Mehagnoul-Schipper et al., 2002) but [O<sub>2</sub>] saturation has been shown to correlate well with CBF measurements as well as with the BOLD signal (Alderliesten et al., 2014) and strong correlations have also been found between [O<sub>2</sub>Hb] and the fMRI signal (Okamoto et

al., 2004; Strangman et al., 2002). Furthermore, a correlation between [tHb] and the BOLD fMRI signal has also been observed (Hess, Stiller, Kaulisch, Heil & Scheich, 2000; Strangman et al., 2002; Strangman et al., 2006). The test-retest reliability of NIRS has also been shown to be similar to fMRI when examining motor tasks within a single session (Strangman et al., 2006). The majority of studies comparing modalities have focussed on comparing NIRS with fMRI which is logical considering they both assess changes in [HHb] (Perrey, 2008). However, good agreement has also been observed between EEG and fNIRS measurements with fNIRS measuring the haemodynamic response resulting from the electrical activation recorded by the EEG (Butti et al., 2006). Furthermore, NIRS has been shown to more accurately distinguish between levels of workload than EEG (Strait & Scheutz, 2014).

### **2.5.9 Reliability of NIRS for assessing neurological responses**

Research investigating the reliability of measurements using NIRS has elicited mixed results. Plichta et al. (2007b) showed that at a group level the detection of activation changes was highly reproducible even over a 3 week period, a result that was replicated in a group of elderly subjects by Claassen, Colier & Jansen (2006). Kono et al. (2007) found a high replicability for [O<sub>2</sub>Hb] and [HHb] in the prefrontal cortex across four repeated sessions. Reliability for [O<sub>2</sub>Hb] has been demonstrated to be higher than for [HHb] and [tHb] in single subjects (Plichta et al., 2006; Schecklemann et al., 2008) and group responses of [O<sub>2</sub>Hb] have been shown to be stable over time (Plichta et al., 2006). Furthermore, good reproducibility of cerebral blood volume measurements (CBV) has been observed even following reconnection of the device (Van de Ven, Colier, van der Sluijs, Walraven, Oeseburg & Folgering, 2001).

Bhambhani et al. (2006) conducted a review of studies examining reliability of assessment of cerebral blood flow changes using NIRS. The experimental conditions examined include postural changes (Houtman, Colier, Hopman & Oeseburg, 1999; Kurihara, Kikukawa, & Kobayashi, 2003; Mehagnhol-Schipper, Colier & Jansen, 2001), rhythmic handgrip exercises (Bhambhani et al., 2006), altered breathing rates and carbon dioxide levels (Totaro, Barattelli, Quaresima, Carolei & Ferrari, 1998; Wantanabe, Matsuo, Kato & Kato, 2003), and incremental exercise (Koike et al., 2004). Bhambhani et al. (2006) found that there was generally a good reliability of measurements across the studies despite the range of experimental protocols. This is in line with findings of more recent studies who found that, although a range of populations and experimental conditions have been examined test-retest reliability remains fairly consistent across studies (Kono et al., 2007;

Zhang, Zhang, Duan, Ma, Lu & Zhu, 2011). Reliability of NIRS has been indicated to be stronger in the [O<sub>2</sub>Hb] and [tHb] measures than the [HHb] measures, particularly at the individual subject level (Kono et al., 2007; Zhang, Zhang, Duan, Ma, Lu & Zhu, 2011). However, Strangman et al. (2006) found that it was [O<sub>2</sub>Hb] and [HHb] that showed the best reproducibility when looking at intertrial and interindividual measurements.

Conversely, Strangmann et al. (2008) found that reliability was observed at a group level but not at single channel or single subject level and Scheckleemann et al. (2008) found that high variability was observed for some measurements both between and within subjects. The reliability of assessments at the single subject level may be limited by the inexact positioning of probes using measurements derived from the 10-20 or 10-20 systems of EEG positioning (Canning & Scheutz, 2013; Plichta et al., 2007) and it has been suggested that single subject reliability has not yet been achieved (Biallas, Trajkovic, Haensse, Marcar & Wolf, 2012; Kono et al., 2007; Plichta et al., 2006, 2007; Scheckleemann et al., 2008). Even when experimental stimuli are constant NIRS has been shown to exhibit trial to trial variability (Hu, Hong & Ge, 2013). There are a number of possible explanations for this including differences in probe placement (i.e. differences in structural regions sampled), individual subject variability (i.e. effort expended, experimental familiarity), changes in physiological noise (i.e. pulse oscillations, respiration) and different coupling between the optode and the head (Hu, Hong & Ge, 2013; Scheckleemann et al., 2008; Strangman, Boas & Sutton, 2002; Zhang et al., 2011). Reliability of NIRS measurements may also be affected by individual variations in superficial tissue and CSF and by a high signal to noise ratio (Haessinger et al., 2011; Scholkmann et al., 2014; Zhang et al., 2011). Moreover, reliability could be affected by movement artefacts if these are not corrected (Scheckleemann et al., 2008).

## **2.6 The importance of validity and reliability in scientific research**

A robust experimental design needs to allow researchers to draw the conclusion that if all variables other than the variable of interest have been controlled in an experiment, then any observed changes are the result of the intervention (Atkinson & Nevill, 2001). In order to be reasonably certain that this is the case there are two key questions that need to be answered; does the equipment or tool that we are intending to use actually measure the phenomena or response that we are interested in observing (is it valid?), and are the measurements consistent and repeatable (is it reliable?) (Bolarinwa, 2015).

### 2.6.1 Validity and measurement error

The validity of a research tool or technique is a cornerstone of research (Atkinson & Nevill, 1998) and is often discussed in combination with reliability. Black and Champion (1976) defined validity as: “the property of a measure that allows a researcher to say that the instrument measures what he says it measures”(p. 222), in other words there must be certainty that the instrument is measuring what it is designed to and not detecting a change or effect that is unrelated to our experimental manipulations (Atkinson & Nevill, 1998). In their definition Johnston and Pennypacker (1980) link measurement validity to measurement accuracy:

“The goal of any scientific measurement operation or procedure is to arrive at the best possible estimate of the true dimensional quality of a natural phenomenon. To the extent that this goal is achieved it is said that the measurement is accurate or valid. Accuracy or validity of the results therefore becomes the yardstick for gauging the quality of any measurement procedure. For purposes of clarity *accuracy* (or *validity*) may be defined as the extent to which measures approximate values of the true state of nature.” (p. 190)

Therefore, in order to ascertain that a phenomenon or effect observed is accurate it must first be established that the methods by which the data is collected are valid. When talking about validity in research the term internal validity is used to refer to how well the experiment is conducted, or in other words how well potential confounding variables are excluded from the experiment, whereas external validity is focussed on how applicable the results are in the real world (Johnson, 1997). The concept of internal validity includes how well a piece of equipment or instrumentation measures the phenomenon being investigated (Jones & Gratton, 2014) and can be determined in four ways; logical (face) validity, content validity, construct validity and criterion validity (George, Batterham & Sullivan, 2015). For the purposes of experimental research using equipment to collect data, the key type of validity of interest is criterion validity. Criterion validity is defined as “the process by which a new measurement or instrument is compared to a previously validated or criterion measure or instrument” (George, Batterham & Sullivan, 2015, p. 24). In order to establish criterion validity a piece of equipment or test is measured against a test or device that has previously been shown to measure the variable of interest, otherwise known as a ‘gold standard’ (Impellizzeri & Marcora, 2009).

## 2.6.2 Reliability in research

Reliability reflects the level of agreement between measurements and refers to the extent to which measurements can be replicated (Bruton, Conway & Holgate, 2000; Daly & Bourke, 2000; Portney & Watkins, 2009). The reliability of a research tool or device is an important factor in determining the error of the measurement and therefore is a critical component of research (Baumgartner, 1989). Measurement error may be caused by systematic bias (e.g., learning error) or by random error (e.g., biological variations) (Atkinson & Nevill, 1998) and the sum of these is known as total error (Chatburn, 1996). Systematic bias usually reflects the trend in measurements to be different in one direction (positive/negative) and is commonly caused by either a learning effect (Coldwells, Atkinson & Reilly, 1994) or by insufficient recovery periods between tests (Atkinson & Nevill, 1998). The reliability of a measurement is intricately linked to the validity, as a piece of equipment cannot be considered as valid if the data obtained is not reliable (Atkinson & Nevill, 1998).

There are a number of terms that are used interchangeably when discussing reliability in research which include 'repeatability', 'consistency', 'reproducibility', 'stability' and 'agreement' (Atkinson & Nevill, 1998; Black & Champion, 1976; Johnstone & Pennypacker, 1980) and these terms are reflected in definitions of reliability. Black and Champion (1976) discussed reliability in terms of a measuring instrument which can be taken to include a piece of equipment and stated that "The reliability of a measuring instrument is defined as the ability of the instrument to measure consistently the phenomenon it is designed to measure" (p.234). Lehner (1979) talked about reliability in terms of the reproducibility of the measurements, which was also reflected in the definition by Goode & Hatt (1952) that "Reliability (is) the extent to which repetition of the study would result in the same data and conclusions" (p. 153). This notion of being able to obtain the same data or conclusions following repetition is particularly important in human research as results are often influenced by interindividual variability (Mattei, Kozak-Ribbens, Roussel, Le Fur, Cozzone & Bendahan, 2002). This is reflected in the definition derived by Johnston & Pennypacker (1980) who provided a detailed definition of reliability in research:

"Reliability refers to the capacity of the instrument to yield the same measurement value when brought into repeated contact with the same state of nature. Thus, this meaning of reliability is concerned with the stability of measured values under constant conditions." (p.191)

Stability in relation to reliability measurements has been linked to the level of between day variability whereas consistency may be taken to refer to within day variability (Baumgarter, 1989). The importance of reliability measurement in research is to quantify variability and extrapolate the implications of that variability to the ability to answer the research question (Atkinson & Nevill, 2001).

## **2.7 Directions for research**

The above review of the literature has identified gaps in the knowledge which will be investigated in the subsequent five chapters. While Hemond, Brown and Robertson (2010) were able to demonstrate improved skill performance in dual task conditions, this finding has not been replicated, furthermore it is unknown whether the benefits would transfer to a continuous motor task. The question will be addressed by the empirical studies in chapters three and seven. The evidence in support of the benefits of a dual task in skill learning is more robust with several studies observing enhanced skill learning in dual task conditions (Chiou & Chang, 2016; Goh et al., 2012; Roche et al., 2007; Song & Bédard, 2015). However, as with the work of Hemond, Brown & Robertson (2010), these studies all used short duration static computer tasks and consequently any potential benefits of dual task training have yet to be examined in a continuous motor task. Chapter seven will address this gap in the literature by examining the effect of two different dual tasks on motor skill learning and retention.

The neural mechanisms underpinning the observed benefits to skill performance and learning have yet to be established. The benefits of a dual task on novel skill performance and learning may be due to the activation of similar neural networks allowing for a greater availability of resources (Hemond, Brown & Robertson, 2010), or to the establishment of an external focus of attention and optimum level of arousal (Roche et al., 2007; Wulf, 2013). Near infrared spectroscopy is an ecologically valid method of analysing neurological processing by inferring activation from the haemodynamic response (Anderson et al., 2018; Brigadoi et al., 2014; Ferrari et al., 2014). The Artinis Portalite NIRS device is lightweight and mobile and therefore would be a useful tool for assessing the neurological responses to a dual task protocol. However, the validity and reliability of this device for assessing haemodynamic responses to cognitive stimuli has yet to be established. Establishing the validity and reliability of equipment is paramount to the accuracy of the results obtained (Atkinson & Nevill, 2001) and consequently the validity and reliability of the Artinis Portalite for determining haemodynamic responses will be

established in chapters four, five and six before it is used to assess haemodynamic responses to the dual task protocols in chapter seven.

## **Chapter 3: The influence of different dual task modalities on performance of a novel task**

### **3.1 Introduction**

The benefits of dual tasks have previously been reported in relation to motor skill learning (e.g., Goh et al., 2012; Roche et al., 2007), but one study in particular has also indicated that a secondary task which engages similar neural networks to the primary task may enhance performance of the primary task (Hemond, Brown & Robertson, 2010). This initial study will examine the effects of two distinctly different dual tasks on novel skill performance.

#### **3.1.1 The influence of dual tasks on novel skill performance**

Research into the negative effects of dual tasks on novel skill performance has been widespread and findings have been reasonably consistent. When two tasks requiring attention are performed concurrently there is a reduction in the ability of participants to perform one or both tasks, an effect known as dual task interference (DTI) (Chen et al., 2013; Houwink et al., 2013). People are not generally aware of experiencing difficulty in performing two tasks at the same time or of the presence of DTI unless the tasks have elements of physical incompatibility or are particularly mentally demanding even when the interference effect is clear in the performance of the skill (Pashler, 1994a).

The most commonly held view on DTI is that people share limited resources between tasks hence, performing more than one task at a time reduces the capacity available to complete both tasks and consequently impairs performance (Pashler, 1994a). Specific theories of dual task interference are discussed in section 2.3.2. Whilst it is possible to reduce or eliminate DTI with practice (Pellecchia, 2005; Ruthruff, Van Selst, Johnson & Remington, 2006; Schaefer & Lang, 2012), repeated studies have demonstrated impairment of primary task performance when the task is novel, both in cognitive and motor skills (e.g., Beilock, Wieranga & Carr, 2002; Isreal, Chesney, Wickens & Donchin, 1980; Schaefer, 2014; Watanabe & Funahashi, 2018). When a task is novel, performers generally benefit most from a skill focussed environment where attention is directed towards the execution of the required movement (Houwink et al., 2013). For example, a dual task has been shown to have a greater effect on novice than expert performers in a golf putting and football dribbling skill (Beilock, Carr, MacMahon & Starkes, 2002; Beilock, Wierenga & Carr, 2002), however when the football dribbling skill was made more complex by requiring the

performers to use their non-favoured foot, experts also exhibited a reduced ability to perform in dual task conditions (Beilock et al., 2002). In addition to the effects of experience level, DTI is expected to be more pronounced when the two tasks being performed contain similar inputs or require similar responses (Navon and Miller, 1987; Pashler, 1994a).

### **3.1.2 Potential benefits of dual tasks on novel skill performance**

The traditionally demonstrated negative effects of dual tasks on novel skill performance, whilst extensively reported (Alavash, Hilgetag, Thiel & Gießing, 2015; Al-Yahya, Dawes, Smith, Dennis, Howells & Cockburn, 2011; Marti, King & Dehaene, 2015; Pashler, 1994a; Patel, Lamar, Bhatt, 2014; Watanabe & Funahashi, 2014) are not always observed (Medeiros-Ward, Watson & Strayer, 2015; Ruthruff et al., 2003; Watson & Strayer, 2010). It has also been demonstrated that some individuals show a resistance to DTI and are more capable of performing two tasks at the same time (Donohue et al, 2012; Watson & Strayer, 2010). A study by Hemond, Brown and Robertson (2010) showed that a dual task paradigm could have a beneficial effect on novel skill performance. The authors examined the performance of a motor sequencing task where participants were required to respond to the position of a coloured cue on a screen by pressing the appropriate button under two different dual task conditions. In one condition participants were asked to count the number of red cues seen whilst completing the motor sequence task (counting task), and in the other condition participants were asked to learn a sequence of coloured cues (sequence task) whilst simultaneously completing the motor sequence task. In the counting dual task condition, performance of the motor sequencing task was significantly reduced compared with control, however, in the sequence dual task condition, performance of the motor sequencing task was significantly enhanced compared with control. This finding suggests that the presence of a second task that was similar in nature to the primary task aided novel skill performance.

Hemond, Brown and Robertson (2010) explained this result by concluding that it was not the presence or absence of the secondary task that was important in determining performance decrements but rather the nature of the secondary task. They hypothesised that secondary tasks which engage similar neurological processes to the primary task may enhance rather than impair performance due to the greater engagement of neural networks (Hemond, Brown & Robertson, 2010). This view contrasts with traditional thinking

which has suggested that dual task interference is increased when the tasks compete for the same neural resources (Rémy et al., 2010).

### **3.1.3 The mechanisms behind dual task benefits to novel skill performance**

The hypothesis that the involvement of similar neural networks plays a role in facilitating performance in dual task conditions, has been supported by studies which have shown an improvement in novel skill acquisition following dual task training. Goh et al. (2012) reported that audio response tasks had a beneficial effect on motor skill learning which the authors also proposed was due to the engagement of similar neural networks. An alternative explanation was provided by Roche and colleagues (2007) who also observed dual task benefits to novel skill learning. Their proposal was that the presence of a dual task created an optimum sense of arousal and facilitated the maintenance of attention during an otherwise 'boring' primary task. Levels of physiological arousal have been shown to be directly related to the cognitive complexity of the task, with increases in arousal being directly related to increases in task difficulty (Karatekin, 2004). Both optimum arousal level and the maintenance of an external focus of attention have been shown to be influential in skill performance (Karatekin, 2004; Vine, Freeman, Moore, Chandra-Ramanan & Wilson, 2013; Wulf, 2013) and therefore, this may provide a suitable explanation for the dual task benefits observed. As none of the authors have used any neuroimaging or other psychophysiological measurement techniques to elucidate the mechanisms behind the beneficial dual task effects, there is currently no evidence to support either theory.

### **3.1.4 The use of pupillometry to examine psychophysiological responses to dual tasks**

Pupillometry is used to measure the pupillary responses to tasks conditions or interventions (Piquado, Isaacowitz & Wingfield, 2010; Sirois & Brisson, 2014). This technique uses changes in pupil dilation to determine the psychophysiological response to a stimulus (Laeng, Sirois & Gredebäck, 2012; Sirois & Brisson, 2014). Pupil diameter has been shown to increase in response to physiological arousal (Beatty & Lucero-Wagoner, 2000; Bradley, Miccoli, Escrig & Lang, 2008; Nassar, Rumsey, Wilson, Parikh, Heasley & Gold, 2012). In addition, increased pupil diameter has been linked to an increase in mental effort in both single (Alnæs, Sneve, Espeth, Endestad, van de Pavert & Laeng, 2014; Szulewski, Fernando, Baylis, & Howes, 2014; Taylor et al., 2015; van der Wel & van Steenbergen, 2018; Zénon et al., 2014), and dual task conditions (Lisi, Bonato & Zorzi, 2015; Karatekin, Couperus & Marcus, 2004), as well as in response to an elevated attentional load (Kang, Huffer &

Wheatly, 2014; Lisi, Bonato & Zorzi, 2015). Pupil dilation has also been shown to increase proportionally with load across multiple cognitive tasks and therefore is a good indicator of task complexity (Chen & Epps, 2014; Haji, Rojas, Childs, de Ribaupierre & Dubrowski, 2015; van der Wel & van Steenbergen, 2018; Zekfeld, Kramer & Feston, 2011). Changes in pupil diameter have been successfully measured using mobile eye-trackers (Szulewski, Fernando, Baylis, & Howes, 2014) and therefore this is a suitable technique to monitor psychophysiological responses to different dual task conditions.

### **3.2 Aims**

This study addressed the following thesis aims:

1. To examine whether a dual task which is expected to activate similar neurological processes as the primary task could be used to facilitate novel skill performance
2. To determine the psychophysiological mechanisms underpinning dual task effects

The study compares performance and pupil dilation in three different conditions; control, backwards counting (counting backwards in 3s from 300) and audio response (identifying when an audio cue is heard). This study will answer the following research questions:

1. Can a secondary audio response task presented during a continuous novel motor skill improve skill performance?
2. Is pupillometry a suitable technique to determine psychophysiological responses to dual task interventions?

It is hypothesised that enhanced performance will be observed in the audio response condition compared to the control condition and impaired dual task performance will be observed in the backwards counting condition compared to the control condition. It is also hypothesised that pupil dilation will be greatest in the backwards condition and smallest in the control condition.

## 3.3 Methods

### 3.3.1 Participants

Eighteen participants (9 male, 9 female; mean age:  $34.5 \pm 7.24$  years) were recruited to take part in this study using convenience sampling. Advertisements were placed around the university and participants were those who responded and met the inclusion criteria, these were:

#### *Inclusion criteria*

- Male or female
- Aged 18-50

#### *Exclusion criteria*

- Uncorrected impairments to vision
- Uncorrected hearing issues
- Injury to arms or shoulders
- Requiring glasses and not able to switch to contact lenses
- Experience of playing the game used as the primary task

Participants provided written, informed consent to take part in the study (see appendix A) after being provided with a participant information sheet (see appendix B). Ethical approval was obtained from the University of Winchester ethics committee before the commencement of this study.

### 3.3.2 Sample size determination

A sample size of 15 participants was determined to be sufficient to detect significant effects with power at the 0.80 level and an alpha of 0.05 as predicted by G\*power (Faul, Erdfelder, Lang & Buchner, 2007). However, 18 participants were recruited to ensure that the randomised order of conditions was completed an equal number of times and to take account of potential participant drop out. The power determination was based on the results of Roche et al. (2007), using the mean  $\pm$  SD values of condition 1:  $437.2 \pm 6.5$ , condition 2:  $441.2 \pm 6.1$ .

### **3.3.3 Experimental procedure**

Participants attended for testing on four occasions separated by at least seven days to allow decay of retained improvements from the previous session to occur (Thapar & McDermott, 2001). The first session was a familiarisation session where participants were provided with a set of instructions for playing the Xbox Kinect™ (Microsoft, Redmond, Washington) bowling game that was used as the novel task in this study. Participants then completed nine trials of the game, three in control conditions and three in each of the two dual task conditions to ensure that differences in task performances were not due to increased familiarisation with the game in subsequent sessions. On the remaining three visits, participants completed three dual task conditions in a counter-balanced and randomised order. These were: control (no dual task), backwards counting (counting backwards in 3s from 300), and audio-response (responding to an audio-cue).

### **3.3.4 Primary task**

The novel task used in this study was a ten pin bowling game entitled 'Pin Rush' from the Xbox Kinect™ Sports Package. The Xbox Kinect™ system enables players to operate the game using body movements rather than a traditional computer game controller. The Kinect system has also been demonstrated to provide reliable movement detection (Yang, Pu, Li, Li, Fan, & Li, 2014) allowing for replication of the same responsiveness in each condition. The game was displayed on a projector screen (205cm x 152cm) and participants stood behind a line marked on the floor, 2 metres away from the screen and directly in front of the Kinect sensor. The Kinect sensor was placed on a table 73cm off the floor and positioned in line with the centre of the screen.

The purpose of this game was to knock down as many pins as possible within the time allowed. Participants started with 1 minute of time and received an additional 5 seconds for every 30 pins they knocked down. It was possible to see the game score and time remaining throughout the game (top left corner of the screen). At the end of the game participants were also able to view their best score from that session as well as the score from the current game but were not able to view scores from previous sessions or scores from other participants.

The game play was viewed from the perspective of the avatar (see Figure 7), and participants were instructed to use only their dominant hand and to continue playing until the time ran out. Participants had no experience of playing the game used and most (17 out

of 18) had never used the Xbox Kinect™ gaming system. Nine trials of the primary task were completed in each condition, and trials were completed in blocks of three with a five minute break in between each block to minimise effects of fatigue. During each break, ratings of perceived exertion (RPE) and fatigue were taken to ensure that physical fatigue did not impact on results (see appendix C for scales used). Although the bowling game may have contained some elements of familiarity for the participants (as participants who had experience of physical ten pin bowling were not excluded), the actions required to operate the game and the nature of game play (knocking down as many pins as possible within a set time-period) were considered distinct enough from standard ten pin bowling to classify this game as a novel task.



Figure 7: Game play from the perspective of the participant (image taken by researcher during game play)

### 3.3.5 Secondary tasks

Participants completed nine trials in each of three dual task conditions. In the control (C) condition participants completed nine trials of the game with no secondary task. In the backwards counting (BC) condition, participants were required to count backwards in 3s from 300 throughout the duration of each trial, commencing again from 300 at the start of the next trial. In the audio-response condition (AR), participants were required to respond

to an audio cue by saying 'now' each time the cue was detected. The audio cue was 750Hz and played through a standard laptop speaker. The cue was played every 15 seconds throughout the duration of each trial (commencing 15 seconds after the start of the game). The backwards counting task was chosen due to previously established detrimental effects on novel skill performance (Beauchet, Dubost, Aminian, Gonthier, & Kressig, 2005). The audio response task was adapted from one of the tasks used by Goh et al. (2012). The accuracy of the backwards counting and accuracy and response time to the audio cue were recorded throughout each trial. In the backwards counting condition accuracy was determined as correctly saying the next number in the sequence, if the participant stated the wrong number, every subsequent number was identified as incorrect unless they returned to the correct sequence of numbers. In the audio-response condition accuracy was defined as uttering a response to the audio cue. Participants were not made aware of their accuracy scores or given any feedback during the session.

### **3.3.6 Measurements**

Performance was determined as game score in each trial and this was recorded manually by the researcher for each trial. Accuracy of backwards counting was recorded manually throughout each trial. The audio-response trials were recorded on an iPad recording application (TwistedWave voice recorder) and response times and accuracy were analysed using the same programme. RPE measurements were determined using the Borg scale (Borg, 1998) (see appendix E) and physical fatigue was determined using a 10-point fatigue scale (Kim, Jesus Lovera, Schaben, Bourdette & Whitham, 2010) (see appendix F).

### **3.3.7 Pupillometry**

Pupil dilation data was collected using mobile eye-trackers (Sensorimotoric Instruments GmbH, Germany) recording at 60Hz. Full pupil data was unavailable for three participants, so analysis was performed on the data for 16 out of 18 participants. Blinks were identified by plotting the response graph using MATLAB software. The identified blinks were then interpolated from the data using the technique described by Mathôt (2013), specifically, using the equations:  $T1 = T2 - T3 + T2$ ;  $T4 = T3 - T2 + T3$ . Artefacts were then rejected from the data using a Hampel filter (Liu, Hancong, Sirish Shah, & Wei Jiang, 2004). The mean pupil response was determined relative to a 1000ms baseline recorded directly before the commencement of each trial (Mathôt, Fabius, Van Heusden & Van der Stigchel, 2018). The baseline was recorded whilst participants were looking at the projector screen directly prior

to the start of each trial to ensure the light intensity between baseline and trial was matched. Environmental light in the laboratory where testing took place was controlled by keeping windows covered.

### **3.3.8 Data analysis**

A one-way repeated measures ANOVA was used to determine game performance and to examine mean pupil dilation for the left and right eyes with the repeated measures variables being the three different conditions. Backwards counting and audio-response accuracy were recorded as a percentage of responses and audio-response time was averaged over each trial and expressed in seconds. A one-way repeated measures ANOVA was used to determine differences between accuracy and response time between the nine trials in each condition. Greenhouse Geiser corrections were applied if assumptions of sphericity were violated and significant effects were investigated using Bonferroni corrected pairwise comparisons. A paired t-test was used to compare the response accuracy between the BC and AR conditions. The alpha level was set at  $p < 0.05$  for all statistical tests. Data was presented as mean  $\pm$  SD and 95% confidence intervals (95% CI) were also reported. Effect sizes were calculated using partial eta squared ( $\eta_p^2$ ) and were interpreted as: small = 0.01, medium = 0.06, large = 0.14 according to guidelines from Cohen, Miles & Shevlin (2001). All data analysis was completed using SPSS version 22.

## **3.4 Results**

### **3.4.1 Primary task performance**

Statistical analysis revealed a significant main effect for condition,  $F(2,34) = 7.66$ ,  $p = 0.02$ ,  $\eta_p^2 = 0.31$ . As displayed in Figure 8 the BC condition demonstrated a significantly lower score ( $M = 111$ ,  $SD = 33$ , 95% CI [93.78, 127.95]) than the C condition ( $M = 150$ ,  $SD = 50$ , 95% CI [114, 188],  $p = 0.035$ ) and AR conditions ( $M = 156$ ,  $SD = 48$ , 95% CI [127, 186.5],  $p = 0.009$ ). There was no significant difference between performance in the control and AR conditions ( $p > 0.05$ ).

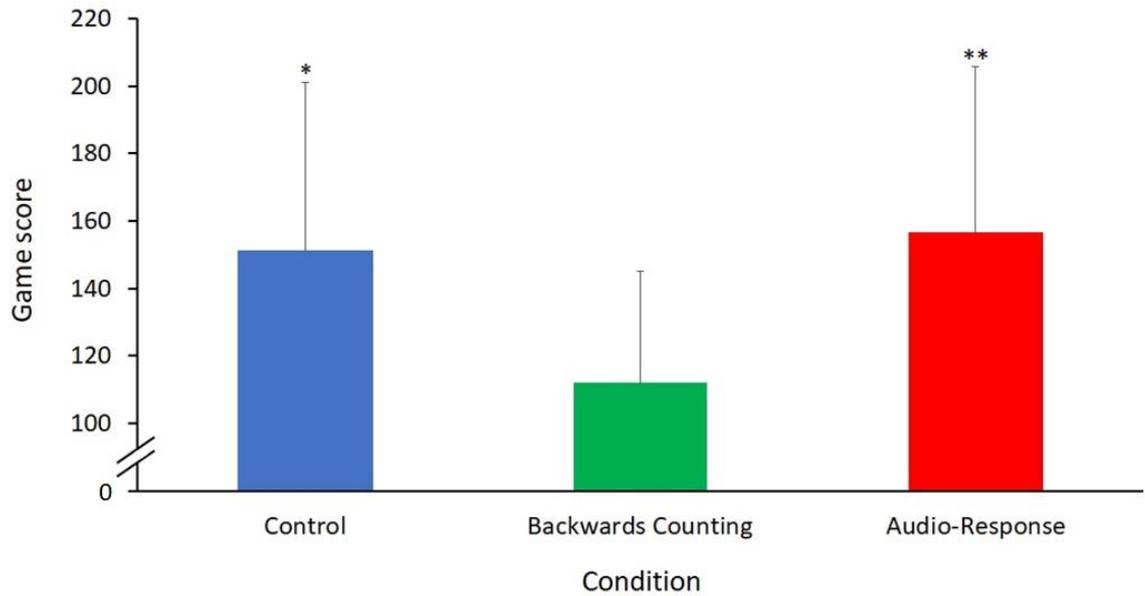


Figure 8: Mean primary task performance in each condition. \* Significantly different from BC ( $p < 0.05$ ), \*\* Significantly different from BC ( $p < 0.01$ ).

### 3.4.2 Secondary task performance

Percentage accuracy of secondary task performance was significantly higher in the AR condition ( $M = 99.79$ ,  $SD = 0.87$ ,  $t = -5.30$ , 95% CI [-17.73, -7.64],  $p < 0.001$ ) than the BC condition ( $M = 87.11$ ,  $SD = 10.23$ ). There was no significant change in backwards counting accuracy,  $F(5.3, 90.12)$ ,  $p = 0.31$ ,  $\eta_p^2 = 0.07$  or audio-response accuracy,  $F(3.46, 58.87)$ ,  $p = 0.14$ ,  $\eta_p^2 = 0.10$  across trials.

### 3.4.3 Pupillometry

There were no significant differences in mean pupil diameter relative to baseline ( $p > 0.05$ ) between the three conditions (see Table 1) in the left eye,  $F(2, 30) = 0.42$ ,  $p = 0.42$ ,  $\eta_p^2 = 0.06$  or the right eye,  $F(2, 30) = 0.57$ ,  $p = 0.57$ ,  $\eta_p^2 = 0.04$ .

Table 1: Mean pupil dilation (cm) in each dual task condition expressed as change from baseline

<i>Pupil dilation (cm)</i>	Control		BC		AR	
	Mean	SD	Mean	SD	Mean	SD
Left eye	0.15	0.37	0.04	0.25	0.14	0.20
Right eye	0.09	0.32	0.04	0.26	0.12	0.25

#### 3.4.4 Fatigue and perception of effect

No significant differences were observed between trials or between sessions for fatigue and RPE ( $p > 0.05$ ).

### 3.5 Discussion

This study aimed to determine whether a simple audio response task presented during the execution of a continuous motor task would aid performance of that skill. The study also aimed to determine whether pupillometry was a suitable technique to examine the psychophysiological responses to a dual task paradigm. The principle finding from this study is that performance of a novel motor skill was impaired by performance of the BC secondary task, but not by performance of the AR secondary task, however, the novel skill performance was also not improved by the presence of the AR task relative to control. Therefore, the findings of this study do not support the work of Hemond, Brown and Robertson (2010) as no positive effect of a dual task on novel skill performance was found. Consequently, the hypothesis of improved performance in the AR condition must be rejected. Furthermore, the use of pupillometry was unable to distinguish between dual task conditions and therefore the hypothesis of greater pupil dilation in the backwards counting must also be rejected.

#### 3.5.1 The elimination of dual task interference

The findings of this study did not demonstrate an improvement in novel skill performance in response to the dual task as has previously been found (Hemond, Brown & Robertson, 2010). It is likely that dual task enhancement to performance was not demonstrated in this study due to a higher task similarity in previous work where primary and secondary tasks both included observation of a sequence of visual cues on a computer screen (Hemond,

Brown & Robertson, 2010). The audio-response task used in this study has been suggested to engage similar neurological processes to the execution phase of a motor task (Goh et al., 2012), but this task has only been used to enhance novel skill learning rather than novel skill performance and thus the similarity of neurological processes may be lower than in tasks previously used to demonstrate performance gains. The primary task used in this study was also of a much longer duration than tasks previously used and involved a continuous skilled movement task whereas previous tasks have been discrete static tasks performed on a computer (Goh et al., 2012; Hemond, Brown & Robertson, 2010). Discrete and continuous tasks have different cognitive demands as discrete tasks require only short periods of focus whereas continuous tasks require more sustained attentional focus (Lee & Genovese, 1989; Wickens, 1991). Attentional focus has been shown to decrease with time on task, consequently, this effort to maintain attention increases the task complexity (Ariga & Lleras, 2011; Langner & Eickhoff, 2013).

Dual task practice often leads to a reduction or elimination of dual task interference (Allen et al., 2009; Ruthruff et al., 2006; Schaefer & Lang, 2012; Strobach et al., 2015), which may be due to a level of automatization of one or both tasks (Logan, 1985; Poldrack et al., 2005; Schaefer, 2014), a movement from serial to parallel processing (Göthe, Oberauer and Kliegal, 2007) or a conversion from declarative to procedural knowledge (Meyer & Kieras, 1997a; Schumacher et al., 2001). Therefore, automatization must also be considered as a potential mechanism to explain the lack of dual task interference observed in the AR condition in this study. This explanation is unlikely, however, as the response to the audio cue was only completed an average of five times per trial (45 times in total during the session), therefore the number of attempts at the secondary AR task would have been insufficient to induce automatization. Moreover, no differences were observed in response times to the audio cue across the trials, which would suggest that this task had not been automatized.

### **3.5.2 Dual task effects of different secondary tasks**

DTI in novel skill performance has been widely established (Beilock, Wieranga & Carr, 2002; Chen et al., 2013; Houwink et al., 2013; Schaefer, 2014) and this effect has been attributed to either a response selection bottleneck (Pashler, 1994a) or a limitation in central capacity (Friedman, Polson, Dafoe & Gaskill, 1982; Tombu & Jolicœur, 2003). Theories of response selection bottlenecks propose that processing systems within the brain are only capable of working on one stimulus at a time (Hommel & Eglau, 2002; Pashler, 1994a,b; Schumacher

et al., 2001) particularly at the stage of response selection (Navon & Miller, 1987). Responses for multiple stimuli requiring processing from similar neural networks occur serially rather than in parallel, and a suspension of response processing for a second task occurs until the response selection for the first task has been made (Navon & Miller, 1987) leading to a 'bottleneck' in response selection.

The different secondary task responses observed in this study could be attributed to a difference in task complexity with the more challenging task (counting backwards) causing an increase in central capacity demands due to an increase in mental effort (Tomblu & Jolicœur, 2003). While task complexity is not normally a determinant factor of the presence of DTI (Brown & Bennett, 2002; Navon & Miller, 1987), the AR task used in this study did not require any working memory capacity or present a choice of responses, it would not ordinarily be expected to induce DTI (Pashler, 1994a), although the expectation of a secondary task alone has been shown to induce DTI (Gottsdanker, 1979; Logan & Gordon, 2001). However, the most likely explanation for the results found in this study is that the effect of this task was to facilitate an external focus of attention as participants listened for the presentation of the audio cue. An external focus of attention is beneficial for skill performance (Wulf, 2013), which would explain the fact that over half the participants recorded their highest average score in this condition.

### **3.5.3 Pupillometry**

Unlike the performance measures the pupil dilation data did not detect any differences between tasks. This is at odds with previous literature as pupil diameter has been shown to increase from baseline in response to the cognitive demands of both single and dual task conditions (Alnæs et al., 2014; Jainta & Baccino, 2010; Karatekin et al., 2004; Lisi et al., 2015; Takeuchi, Puntous, Tuladhar, Yoshimoto & Shirama, 2011). Pupil dilation has been shown to increase both as a result of increased mental effort and increased physiological arousal (Bradley, Miccoli, Escrig & Lang, 2008; Nassar, Rumsey, Wilson, Parikh, Heasley & Gold, 2012; Takeuchi et al., 2011).

The lack of effect of the dual tasks on pupil dilation in the current study can be explained in a number of ways. First, it is feasible that the lower performance in the BC condition may indicate a division of cognitive resources rather than an additive effect of the secondary task (O'Shea, Morris & Iansek, 2002; Verhaeghen, Steitz, Sliwinski & Cerella, 2003). Consequently, the increased demands of the secondary task would not lead to an increase in mental effort reflected in a change in pupil dilation. This potential explanation is

in line with previous research that has demonstrated that during periods of high cognitive activity there is a separation of external task related attention from internal processing which is reflected in pupil dilation (Smallwood et al., 2011). In the AR condition the lack of increase in pupil dilation compared to control combined with no decrement in performance suggests that the AR task did not produce any additional cognitive demands compared to control. Although very simple dual tasks have been shown to impair novel task performance, the absence of a choice response requirement in the AR task means this task was most likely not sufficiently demanding to elicit DTI (Pashler, 1994b). However, the requirement of a response would have been expected to induce some additional cognitive demands so the absence of a difference in pupil dilation between the C and AR conditions is also unexpected. This finding is however, in line with the work of Karetakin, Couperus and Marcus (2004) who failed to find pupil diameter changes in response to a dual task paradigm.

An alternative explanation for the lack of effects may be due to the method of recording the data. Pupil dilation is affected by changes in light (Binda & Gamlin, 2017; Mathôt & Van der Stigchel, 2015), as well as by movements, including movements of the eye during visual search (Mathôt, Dalmaiger, Grainger & Van der Stigchel, 2014; Mathôt, van der Linden, Grainger & Vitu, 2015). Although a filter was used on the data the influence of light and movement on the data may have remained. Blinds were used in the room to eliminate external light, however, the nature of the primary task meant that light coming from the screen was constantly changing which could have had a substantial effect on the data. Moreover, the participants were constantly moving and eye saccades were most likely large to take in all the different information on the screen which would also have affected the data (Mathôt et al., 2014). These factors taken together mean it is difficult to establish whether the lack of differences between the data is due to a physiological mechanism or measurement error. Consequently, it appears that pupillometry is not a suitable method for determining the mechanisms behind dual task effects on performance of a motor skill where movement is required.

#### **3.5.4 Real world implications of understanding different dual task effects**

Understanding the effects of a similarity based dual task on performance during a continuous movement task is applicable to real-world situations. As dual tasks are often used as a determinate of automaticity in skill performance (Gabbett, Wake & Abernathy, 2011; Logan, 1985; Poldrack et al., 2005), understanding different effects of dual tasks on

skill performance may change the understanding of skill acquisition. If DTI during novel task performance varies depending on task type as has been demonstrated in this study, then ability to perform a skill in dual task conditions may not infer learning but rather alterations in dual task effects. These findings may also have particular implications for clinical populations such as stroke survivors, or those suffering from brain injury, where dual task capability is often used as a determinant of impairment/recovery (Kizony, Levin, Hughey, Perez & Fung, 2010; Rochester, Galna, Lord & Burn, 2014; Taylor, Delbaere, Mikolaizak, Lord & Close, 2013). Dual task ability is commonly used as an assessment tool to determine risk of falls (Montero-Odasso, Muir & Speechley, 2012; Nordin, Moe-Nilssen, Ramnemark & Lundin-Olsson, 2010), severity of concussion (Lee, Sullivan & Schneiders, 2013), and more recently in diagnosis of dementia (Ceide, Ayers, Lipton, & Verghese, 2018; Montero-Odasso et al., 2017; Nielsen, Simonsen, Siersma, Hasselbalch & Hoegh, 2018). In these situations, a difference in DTI in response to difference tasks may cause incorrect judgements of functional ability or recovery to be made.

The findings of this study have interesting implications for learning in dual task conditions. Practicing under dual task conditions has been shown to enhance long-term retention of a skill even when initial skill performance is impaired (Malone & Bastien, 2010). Moreover, it has been shown dual tasks that engage similar neural networks to the primary task can enhance learning of short duration, static, computer based, motor skill tasks (Chiou & Chang, 2016; Goh et al., 2012; Roche et al., 2007). The results of this study indicate that these benefits to learning could transfer to longer duration motor skills with a movement component. This finding may have implications, not only for accelerating skill learning for healthy populations in a real-world environment but also for improving clinical outcomes. A number of studies have demonstrated a benefit to conducting stroke rehabilitation under dual task conditions showing improved recovery outcomes using dual tasks with the traditionally observed impairments to novel skill performance (Choi, Lee & Lee, 2014; Kim, Han & Lee, 2014; Plummer, Villalobos, Vayda, Moser & Johnson, 2014; Yang, Chen, Lee, Cheng & Wang, 2007). Therefore, using a dual task which does not impair novel performance may further facilitate these improvements in recovery outcomes.

### **3.5.5 Limitations**

The limitations of the study in relation to the use of pupillometry have been discussed in section 3.5.3 but there are other potential limitations that need to be considered. First, the dual tasks used in this study, whilst chosen to line up with previous

literature had very different task demands and used different response processes (audio response and working memory) which may have limited the comparability of the two tasks. In an effort to reduce the likelihood of overfamiliarity with the secondary task, the audio cue in the AR task was also presented very infrequently (once every 15 seconds), which could have limited the effect of the task on primary task performance. Second, although every effort was taken to avoid learning of the primary task some participants did show a steady improvement in performance across sessions regardless of dual task condition. Although condition order was randomised it is conceivable that there was a learning effect that influenced the results.

### **3.6 Conclusion**

The BC condition significantly impaired novel task performance compared to the control and AR condition, whereas the AR condition did not impair performance of the primary or secondary tasks. The differences in dual task interference effects were not determined by differences in pupil dilation indicating that the neurological mechanisms underpinning dual task interference require further investigation. The findings of this study have provided a useful starting point for the investigation of potential dual task benefits to skill learning and performance. They have not, however, provided any information regarding the neurological mechanisms responsible for DTI and they demonstrate that pupillometry is not a useful tool for understanding these mechanisms. Furthermore, the findings in this study have also indicated that the nature of the dual tasks compared needs to be carefully considered in order to draw useful conclusions about differences in levels of DTI. Both of these issues will be addressed in the remainder of this thesis. The next three chapters will examine the usefulness of a near infrared spectroscopy device to investigate activation of the dorsolateral prefrontal cortex, a region involved in DTI (Leone et al., 2017). The final chapter will then re-examine dual task effects on novel skill performance and look at the effects on skill learning using the NIRS device to attempt to identify the neurological mechanisms responsible for DTI effects.

## **Chapter 4: Absolute and relative methods of analysing near infrared spectroscopy data to determine haemodynamic changes in response to cognitive demands at rest and during exercise**

### **4.1 Introduction**

The previous chapter established that the use of pupil dilation data recorded using mobile eye-trackers did not provide a useful method of distinguishing between the different demands of dual tasks. Single position NIRS presents a potentially suitable method for assessing neurological activation within the prefrontal cortex which is a region which has been indicated as involved in dual task interference (Leone et al., 2017) and consequently could provide useful information on the neurological responses to a dual task protocol. The subsequent three chapters will examine the use of NIRS to assess haemodynamic changes in the prefrontal cortex.

Near-infrared spectroscopy (NIRS) is a non-invasive and ecologically valid method of determining changes in tissue oxygenation (Strangman et al., 2006; van Beekvelt et al., 2002), which uses continuous monitoring of haemodynamic variables such as oxyhaemoglobin [ $O_2Hb$ ] and deoxyhaemoglobin [ $HHb$ ] to infer neurological changes (Strangman et al., 2006). As increases in oxygen delivery are indicative of increases in neurological activation (Plichta et al., 2006; Schecklmann et al., 2008) this method provides an excellent opportunity to examine localised changes in haemodynamics in response to the dual task protocols applied as part of this programme of research. NIRS devices consist of an emitter and a receiver, and in the Artinis Portalite NIRS device used in this study these are both located on a single optode (see Figure 13).

Near infrared light is emitted at a constant frequency and oxyhaemoglobin [ $O_2Hb$ ], deoxyhaemoglobin [ $HHb$ ] and total haemoglobin [ $tHb$ ] are determined by assessing the attenuation of the light returning to the receiver (Ferrari & Quaresima, 2012; Strangman et al., 2006). The Artinis Portalite emits light at two frequencies 760nm and 850nm. Although single position NIRS has been used to assess neurological activation in a number of studies (e.g., Cheung et al., 2014; Debevec & Mekjavic, 2012; Keramidis, Kounalakis, Eiken & Mekjavic, 2012; Oussaidene et al., 2013; Smith & Billaut, 2010), the optimum method of processing NIRS data, as well as the validity and reliability of this method have yet to be established. This chapter will make a comparison of the use of absolute and relative determinations of haemodynamic response to determine which data provides the best

correlation with behavioural responses in during a cognitive test. Absolute and relative techniques for data processing are discussed further in section 4.1.1.

#### **4.1.1 Absolute and relative methods of processing NIRS data**

The single position NIRS used in this study is the Artinis Portalite (Artinis Medical Systems), which is a continuous wave system (cw-NIRS). Continuous wave systems measure changes in [O<sub>2</sub>Hb] and [HHb] using a modified version of the Beer-Lambert law (Ferrari & Quaresima, 2012). The absolute values obtained using this method are then analysed in relation to a baseline value or arbitrary zero. Whilst the arbitrary zero is the most commonly used method of data processing (Porcelli et al., 2010; Subudhi, Dimmen & Roach, 2007; Tempest, Eston & Parfitt, 2014), there are examples of a baseline taken from directly prior to the commencement of the trial being used as a relative value for comparison (Kakimoto et al., 2009; Subudhi, Olin, Dimmen, Polaner, Kayser, & Roach, 2011) and it has not yet been established which of these provides the most accurate assessment of neurological changes. As cerebral blood flow has been shown to fluctuate spontaneously during resting conditions (Hoshi, 2011; Hoshi & Tamura, 1997; Toronov et al., 2000) it is important that any baseline value used for comparison is obtained from directly prior to the start of the trial. As NIRS measurements of cerebral haemodynamic changes can be affected by changes in blood flow induced by physical exercise (Robertson & Marino, 2016; Thomas & Stephane, 2008; Yanagisawa et al., 2010) it is also important to determine whether the same method of data processing is accurate during exercise.

The cw-NIRS systems assume consistent tissue properties to determine the absolute values obtained (Ferreira, Hueber & Barstow, 1985; Patterson, Chance & Wilson, 1989) and whilst it has been demonstrated that these values provide an accurate measure of [tHb] changes in the calf muscle (Stone et al., 2016), it is unknown whether these values would provide a valid assessment of haemodynamic changes in the prefrontal cortex.

#### **4.1.2 The use of a Stroop protocol in cognitive testing**

Although the eventual use of the NIRS system will be to assess prefrontal cortex responses to a dual task protocol, in order to establish the optimum method of data processing as well as the validity and reliability of this technique it is important to use a cognitive task that has already been demonstrated to activate the prefrontal cortex. Whilst the prefrontal cortex has been indicated as a region of interest in dual task studies (Leone et al., 2017) and is consequently of interest in the examination of the effects of dual tasks on skill learning

and performance, there is no consistent evidence base for the involvement of this region in dual task interference and therefore an alternative task must be used. The Stroop colour word task is suitable for this purpose as it has been consistently demonstrated to activate the prefrontal cortex (Schroeter et al., 2002; Yanagisawa et al., 2010). Moreover, this task requires the maintenance of attentional focus (Bench et al., 1993) meaning that the region involved in the completion of this task is likely to be the same as that involved in the completion of a dual task.

The Stroop colour-word task (Stroop, 1935) has three variations of trials; congruent, where the colour and word match (e.g., the word red written in the colour red); incongruent, where the colour and word do not match (e.g., the word red written in the colour blue) or neutral where the word is unrelated to the colour (e.g., lot) (Zysset, Müller, Lohmann & von Cramon, 2001). The strongest neurological response is expected to be in the incongruent trials where there is a disparity between the colour and the word and the weakest response in the congruent trials where the colour and word match (Duncan-Johnson & Kopell, 1981, Milham et al., 2001). It is expected that the lower interference effect in congruent trials is due to a more automatic processing of word stimuli (MacLeod, 1991). In other words, we are more familiar with reading a word than naming the colour of the word.

There is no consensus as to which prefrontal region is responsible for the Stroop effect. Without access to detailed brain mapping the most effective way to identify a localised region of the brain is to use the 10-20 method of positioning (Jasper, 1958) which involves measuring external cranial landmarks to determine the location of different regions of the brain. According to the 10-20 positioning system previous studies have indicated a role of the Fp1/Fp2 positions (Sakatani, Xie, Lichty, Li & Zuo, 1998; Tanida, Sakatani, Takano & Tagai, 2004; Tsujii, Komatsu & Sakatani, 2013), and the AF3/AF4 positions (Tanida, Katsuyama & Sakatani, 2007; Thomas & Stephane, 2007; Zhai, Li, Zhang & Gong, 2009) in response to the Stroop protocol, all of which are located over the frontal regions (on the forehead). As there is no consensus in the literature as to which frontal region is involved in the Stroop interference effect, in order to assess the methods of analysis available it is important to examine all of these regions to determine which one has the strongest link to task performance when using the cw-NIRS device.

## 4.2 Aims

The aims of this study were to induce prefrontal cortex activation using the Stroop colour word test and: 1) Determine the optimum way of processing the data obtained by the Artinis NIRS device at rest and during exercise; 2) Identify the optimum positioning of a single position NIRS device to determine neurological activation in the prefrontal cortex.

Therefore, the research questions addressed in this study were:

1. Does absolute data provide a useful determinant of haemodynamic responses to a cognitive stimulus at rest and during exercise?
2. Which method of relative data processing provides the most accurate determinant of the haemodynamic response to a cognitive stimulus at rest and during exercise?
3. Which region of the prefrontal cortex is activated in response to the Stroop colour word task?

It was hypothesised that absolute data would not provide useful information about the haemodynamic response and that the optimum way of processing the data would be to use an arbitrary zero. It was also hypothesised that the haemodynamic response would be strongest in the left side of the prefrontal cortex (Fp1/AF3) positions.

## 4.3 Methods

### 4.3.1 Participants

Fifteen healthy participants (10 male, 5 female; mean age:  $25.5 \pm 4.72$  years; stature:  $1.76 \pm 0.8$  m; body mass:  $74.17 \pm 13.37$  kg) were recruited to participate in this study using convenience sampling. Participants were identified from a sample of university students and staff and were included in the study if they met the following criteria:

#### *Inclusion criteria*

- Male or female
- Aged 18-40 years

#### *Exclusion criteria*

- Colour blind
- Suffering from any physical illness that would preclude maximal exercise testing (e.g., high blood pressure, heart disease)
- Suffering from any injury that would prevent cycling at maximal intensity

Institutional ethical approval was obtained prior to the commencement of data collection and participants provided written consent (see appendix A) to participate after being provided with a participant information sheet (see appendix B). Participants were instructed to avoid exercise for 24 hours prior to each session and to arrive at the laboratory in a fully rested and hydrated state no less than 3 hours postprandial.

#### **4.3.2 Sample size determination**

A sample size of 15 was determined to be sufficient to detect power (0.80) at an alpha level of  $p < 0.05$  as predicted by G\*power (Faul, Erdfelder, Lang & Buchner, 2007), based on the mean  $\pm$  SD values of condition 1:  $9.2 \pm 1.1$ , condition 2:  $5.7 \pm 0.6$  in the paper by Shibuya, Tanaka, Kuboyama and Ogaki (2004) examining cerebral oxygenation changes during exercise.

#### **4.3.3 Experimental procedure**

Participants attended four testing sessions over a 2-4 week period with a minimum of 48 hours between visits. Participants completed a graded exercise test (GXT), a familiarisation trial and two NIRS assessment trials which consisted of three resting Stroop tests and three exercise Stroop tests completed at an intensity of 90% of the gas exchange threshold (GET). Trials were conducted at the same time of day ( $\pm 2$  hours) and took place in a temperature controlled laboratory.

#### **4.3.4 Graded exercise test and determination of gas exchange threshold (GET)**

During the initial testing session participants completed a graded exercise test (GXT) using a ramp protocol which is a traditional method of determining maximal oxygen uptake (Barker, Williams, Jones & Armstrong, 2011; Buchfuhrer, Hansen, Robinson, Sue, Wasserman & Whipp, 1983; Chin et al., 2011). The test was completed on an electronically braked cycle ergometer (SRM Ergometer, Jülich, Germany) and breath by breath data was collected using an online gas analyser (Cortex Biophysik, Leipzig, Germany). Participants commenced the test by cycling for five minutes with resistance set at 0 watts (W), which was used as a warm-up period. Following this five minute period the cycle ergometer increased the pedal resistance by 1 W every 3 seconds. Participants were instructed to maintain a cadence of  $\sim 75$  revolutions per minute (rpm) and to continue cycling until they could no longer maintain this cadence. Pedal frequencies of between 60-80 rpm are considered preferable by untrained cyclists (McKay & Bannister, 1976) and pilot testing

confirmed that on the SRM ergometer participants preferred a cadence at the upper end of this range. The test was concluded either by the participant determining they could no longer continue or by the researcher instructing the participant to stop due to cadence dropping below 70 rpm. Cadence, pedal resistance and duration of test were all displayed on a computer monitor placed on a table directly in front of the cycle ergometer. When using a ramp protocol a plateau in oxygen uptake ( $\text{VO}_2$ ) is not always identified (Rossiter, Kowalchuk & Whipp, 2006), therefore peak  $\text{VO}_2$ , respiratory exchange ratio (RER) and maximum heart rate were recorded as recommended in the literature (Howley, Bassett & Welch, 1995; Midgley, McNaughton, Polman & Marchant, 2007). Heart rate was recorded using a chest strap and watch (Polar Electro UK Ltd., Warwick, England).

#### **4.3.5 Determination of gas exchange threshold (GET)**

The GET was determined for each participant from a graph of the  $\text{VO}_2$  response. The GET (also referred to as ventilatory threshold (VT)) marks the point during exercise at which aerobic energy production is supplemented by anaerobic energy production (Wasserman, 1984) and 90% GET has been shown to represent a moderate intensity exercise domain (Brittain, Rossiter, Kowalchuk & Whipp, 2001; Jones & Poole, 2013). The GET was determined using the modified V-slope method (Beaver, Wassermann & Whipp, 1986; Davis, 1985). This method of determination involves plotting the volume of oxygen ( $\text{VO}_2$ ) against the volume of carbon dioxide ( $\text{VCO}_2$ ), the GET is then marked as the  $\text{VO}_2$  value at the point at which there is an increase in the slope of the plot (see Figure 8) (Gaskill, Ruby, Walker, Sanchez, Serfass & Leon, 2001). Each graph was visually inspected independently by two reviewers and the GET agreed. Although the modified V-slope method of determining the GET has been shown to be valid (Wasserman, Beaver & Whipp, 1990), it involves visual inspection and identification by the researcher and consequently can be subject to human error, therefore, it is best practice for each plot to be examined by more than one researcher (Gaskill et al., 2001). Following determination of the GET a work rate equal to 90% GET was determined and this work rate was used as the exercise intensity

during the exercise Stroop.

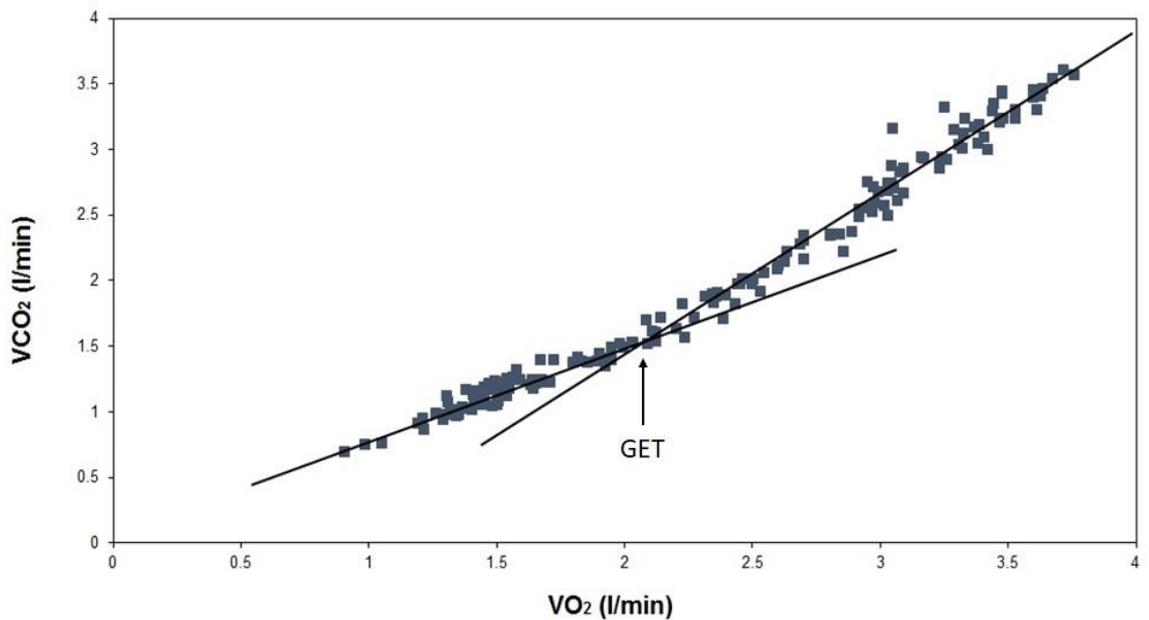


Figure 9: Typical VO<sub>2</sub> vs. VCO<sub>2</sub> graph showing lines used to determine GET by visual inspection.

#### 4.3.6 Stroop test protocol

The Stroop test protocol involved the presentation of one stimulus every 2 seconds. Each stimulus was preceded by a 300ms fixation cross, followed by presentation of the stimulus (word) for 1200ms, and finally a 500ms interval before the commencement of the next trial. These timings were based on the Stroop test protocol described by Milham et al. (2001). The Stroop protocol consisted of one block of 36 congruent trials (e.g., the word red written in the colour red), one block of 30 neutral trials (e.g., the word lot written in any colour), and one block of 36 incongruent trials (e.g., the word red written in the colour blue) (see Figure 9).



Figure 10: Example Stroop stimuli in the congruent, neutral and incongruent conditions

Each Stroop test therefore consisted of 102 trials and took approximately 3.5 minutes to complete. Whilst the neutral trials were always presented second, the order of the congruent and incongruent trials were counterbalanced across participants and across sessions. A wireless keyboard was used for completion of the Stroop test with coloured stickers situated on six keys for each colour (see Figure 10). The test was designed using the Psychopy opensource software (Peirce, 2007) and completed on a Sony VAIO laptop with a 17" screen. Participants were seated 170cm from the laptop during all trials and the screen brightness was kept at a consistent level.

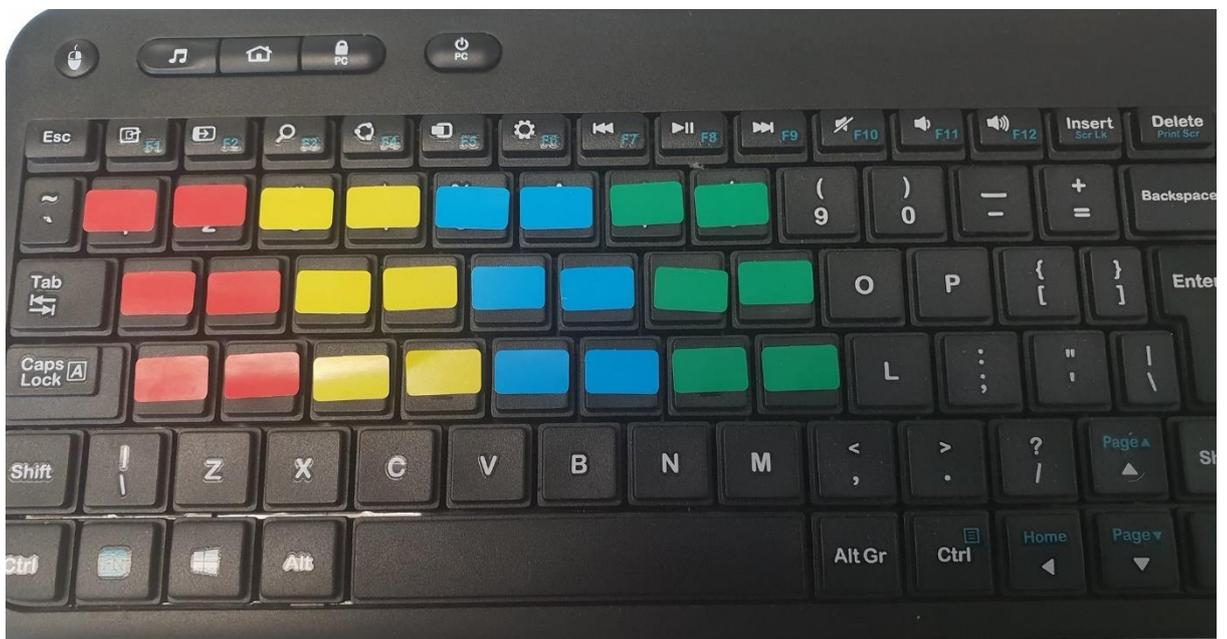


Figure 11: Wireless keyboard used for Stroop protocol with coloured stickers attached

#### 4.3.7 Familiarisation trial

Participants were familiarised with the Stroop protocol in order to minimise any learning effects during the NIRS trials. During the familiarisation trial five Stroop tests were completed at rest for familiarisation with the Stroop protocol and one Stroop test was completed on the bike for familiarisation with the 90% GET work rate and the process of completing a Stroop test on the bike. During the familiarisation session participants were also measured for the NIRS probe placements using the 10-20 positioning system (Jasper, 1958) (see Figure 12). Full details of the measurements used to position the probes are detailed in appendix D.

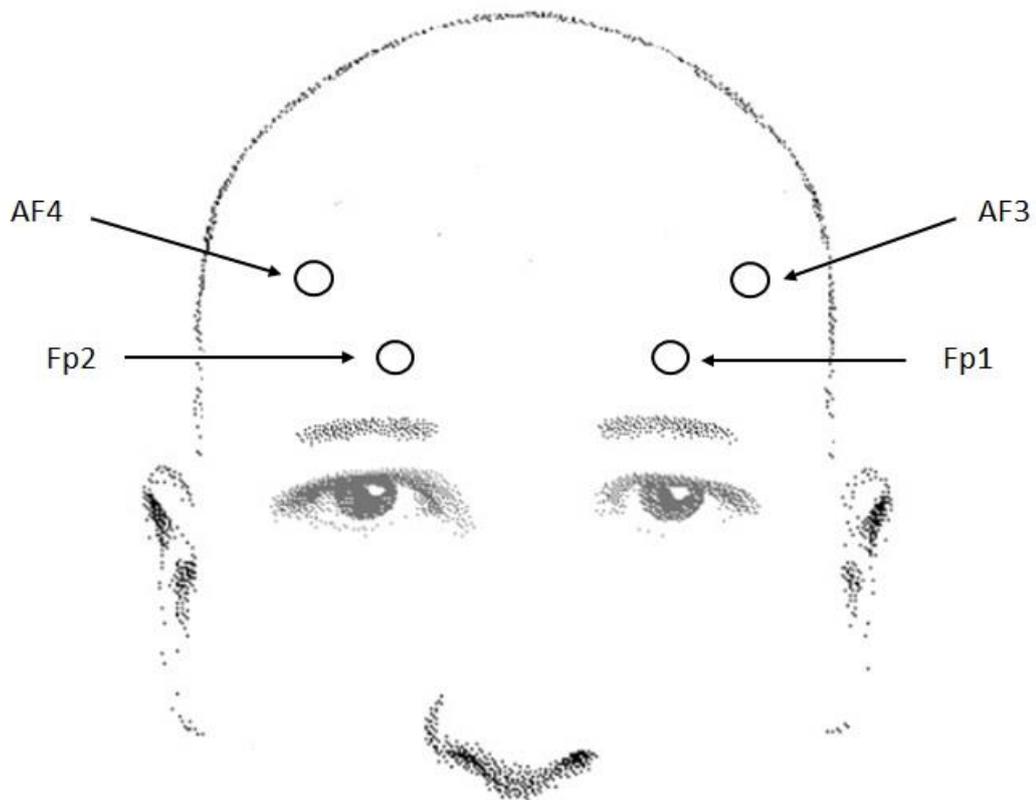


Figure 12: Locations used for NIRS probe placements

#### 4.3.8 Experimental trials

The same protocol was followed in both of the experimental trials. At the commencement of the trial the participants remained seated for five minutes whilst resting HR was measured and NIRS probes were positioned in either the Fp1 and Fp2 positions or the AF3 and AF4 positions (see Figure 3). NIRS probes were affixed to the correct position using bi-adhesive tape and covered with a crepe bandage and bandana to reduce probe movement and minimise external light (Bailey, Vanhatalo, Wilkerson, DiMenna & Jones, 2009; Canning & Scheutz, 2013; Hoshi et al., 2005). The same NIRS probe was used for the same position across participants. Once NIRS positioning was completed participants completed three Stroop tests at rest and three Stroop tests during exercise (see Figure 12). Resting Stroop tests were separated by five minutes of rest and exercise Stroop tests commenced five minutes into a ten minute bout of cycling at 90% GET. Participants assumed an upright seated position on the bike and the keyboard was held in a suitable position by the primary researcher. During completion of the Stroop test participants maintained a cadence of >70

RPM. Following each exercise bout participants rested for a minimum of ten minutes or until HR returned to resting levels ( $\pm 10$  bpm). This resting period was chosen to ensure that physiological responses to exercise had returned to resting levels as cerebral blood flow can take up to 8 minutes to return to baseline following a bout of exercise (Byun, Hyodo, Suwabe, Kujach, Kato & Soya, 2014)

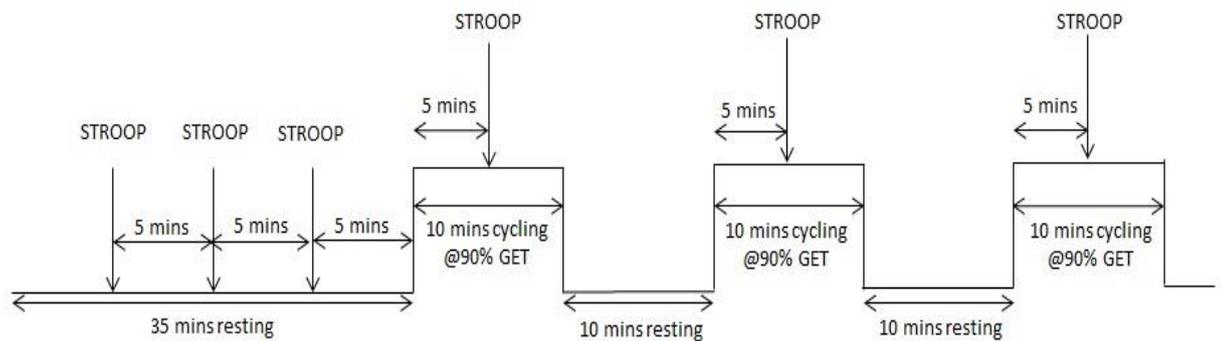


Figure 13: Study protocol for NIRS sessions including STROOP tests at rest and during exercise at 90% GET

#### 4.3.9 NIRS Data collection

Haemodynamic changes in response to the Stroop protocol were continuously monitored during the NIRS trials using the Artinis Portalite NIRS device (Artinis medical systems, Einsteinweg, The Netherlands). The Artinis Portalite system consists of one probe with 3 LED optodes and one receiver (see Figure 13). The optodes are positioned at 30 mm, 35mm and 40 mm respectively and each emits light at two wavelengths (760 nm and 850 nm). The probe measures 58x28x6mm and it is attached to the battery pack by a 1.3m wire. Each battery pack measures 83x50x20mm and weighs 84g. The signal was transmitted via bluetooth to a personal computer and recorded using Oxysoft software (Artinis Medical Systems, Einsteinweg, The Netherlands). This software uses spatially resolved spectroscopy (SRS) where the intensity profile of spatial light is assessed as a function of distance of light from the emitter with the assumption of constant light scatter (Stone et al., 2016) to determine values for haemoglobin concentration and tissue saturation. The midpoint of the probe was situated over the relevant position (e.g., Fp1 or Fp2) to ensure the characteristic ‘banana shaped’ profile of light propagation (Ehlis et al., 2005; Gratton, 1994;

Haessinger et al., 2011) included the region of interest. A sampling rate of 50 Hz was used during all trials.



Figure 14: Artinis Portalite NIRS device (image from [www.artinis.com](http://www.artinis.com))

#### **4.3.10 Data analysis**

##### *Behavioural Data*

Percentage accuracy (ACC) and response time (RT) during the Stroop test was recorded and averaged for congruent, neutral and incongruent blocks during resting and exercise trials. Where the participant failed to record a response during the 1200ms stimulus presentation a response time of 1.2 s was recorded and the trial was marked as an incorrect response. A 2 (trial) x 2 (resting state) x 3 (Stroop block) repeated measures ANOVA was used to compare Stroop response time (RT) between the AF3/AF4, Fp1/Fp2 trials at rest and during exercise and to compare response times between the congruent, neutral and incongruent trials. A 2 (trial) x 2 (resting state) x 3 (Stroop block) repeated measures ANOVA was also used to compare Stroop response accuracy (ACC). Greenhouse Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. A Pearson's correlation test was also used to determine relationships between Stroop data and  $[O_2Hb]$ ,  $[HHb]$  and  $[tHb]$  values recorded by the NIRS using the different methods of processing.

### *NIRS Data analysis*

Mean [O<sub>2</sub>Hb], [HHb] and [tHb] values were determined for congruent, neutral and incongruent trials and averaged for resting and exercise trials for in each frontal position. Two different techniques were used to determine relative changes. The first technique used a 1000ms baseline taken from directly prior to the start of each trial (described as baseline) deducted from the mean trial values, and the second involved setting the initial data point of each trial to an arbitrary zero and determining the change from this point (described as zero). Absolute values recorded by the Artinis oxymon software were also analysed (described as absolute). A 4 (position) x 2 (rest) x 3 (block) repeated measures ANOVA was used for each chromophore to determine whether there were differences in each position using the different methods of processing at rest and during exercise. Greenhouse Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. Separate paired t-tests were used to interrogate significant interactions between rest and position and between rest and block and a separate one-way repeated measures ANOVAs were used to examine significant interactions between position and block.

The alpha level for all data analysis was set at  $p < 0.05$ . Data was presented as mean  $\pm$  SD and 95% confidence intervals (95% CI) were also reported where appropriate. Effect sizes were calculated using partial eta squared ( $\eta_p^2$ ) and were interpreted as: small = 0.01, medium = 0.06, large = 0.14 according to guidelines from Cohen, Miles and Shevlin (2001).

## **4.4 Results**

### **4.4.1 Behavioural data (Stroop)**

#### *Response Time (RT)*

Analysis of Stroop RT revealed a significant main effect for Block,  $F(1.26, 17.59) = 32.34, p < 0.001, \eta_p^2 = 0.70$ . Post hoc comparisons showed that RT was significantly quicker in the congruent blocks than in the neutral ( $p = 0.002$ ) or incongruent ( $p < 0.001$ ) blocks. RT was also significantly quicker in the neutral blocks than the incongruent blocks ( $p < 0.001$ ) (see Table 2). There was no significant main effect for Trial,  $F(1,14) = 0.22, p = 0.65, \eta_p^2 = 0.02$  or Rest,  $F(1,14) = 1.73, p = 0.21, \eta_p^2 = 0.11$ . There were no significant Trial x Rest interactions,  $F(1,14) = 0.50, p = 0.49, \eta_p^2 = 0.04$ , Trial x Block interactions,  $F(2,28) = 0.70, p = 0.51, \eta_p^2 = 0.05$  or Rest x Block interactions,  $F(2,28) = 1.86, p = 0.17, \eta_p^2 = 0.12$ .

### Accuracy (ACC)

Stroop ACC data also revealed a significant main effect for Block,  $F(2,28) = 5.10, p = 0.01, \eta_p^2 = 0.27$ . Pairwise comparisons revealed that responses were significantly more accurate in the congruent blocks than the incongruent blocks ( $p = 0.039$ ) (see Table 2). No significant main effects were observed for Trial,  $F(1,14) = 0.44, p = 0.52, \eta_p^2 = 0.03$ , or Rest,  $F(1,14) = 3.47, p = 0.08, \eta_p^2 = 0.20$ . There were no significant interactions for Trial x Rest,  $F(1,14) = 0.39, p = 0.51, \eta_p^2 = 0.03$ , Trial x Block,  $F(2,28) = 2.05, p = 0.15, \eta_p^2 = 0.13$ , or Rest x Block,  $F(2,28) = 2.78, p = 0.08, \eta_p^2 = 0.17$ .

Table 2: Stroop response time (RT) and percentage accuracy (ACC) for congruent and incongruent blocks during the AF3/AF4 trials and Fp1/Fp2 trials at rest and during exercise. Values are presented in mean  $\pm$  SD

Trial	Congruent		Neutral		Incongruent	
	RT (s)	ACC	RT (s)	ACC	RT (s)	ACC
<i>Resting</i>						
AF3/AF4	0.52 $\pm$ 0.06	0.95 $\pm$ 0.02	0.56 $\pm$ 0.05	0.95 $\pm$ 0.04	0.60 $\pm$ 0.05	0.93 $\pm$ 0.04
Fp1/Fp2	0.52 $\pm$ 0.05	0.95 $\pm$ 0.04	0.56 $\pm$ 0.05	0.96 $\pm$ 0.03	0.59 $\pm$ 0.05	0.94 $\pm$ 0.05
<i>Exercise</i>						
AF3/AF4	0.52 $\pm$ 0.08	0.94 $\pm$ 0.03	0.57 $\pm$ 0.06	0.94 $\pm$ 0.04	0.61 $\pm$ 0.07	0.93 $\pm$ 0.04
Fp1/Fp2	0.52 $\pm$ 0.06	0.96 $\pm$ 0.03	0.58 $\pm$ 0.07	0.93 $\pm$ 0.05	0.60 $\pm$ 0.07	0.92 $\pm$ 0.05

### 4.4.2 Absolute NIRS data

Examination of [tHb] values using a repeated measures ANOVA revealed significant main effects for Position,  $F(3,42) = 5.09, p = 0.004, \eta_p^2 = 0.27$  and Block,  $F(1.26,17.70) = 15.89, p < 0.001, \eta_p^2 = 0.53$ . No significant main effects were observed for Rest,  $F(1,14) = 2.73, p = 0.12, \eta_p^2 = 0.16$ . Pairwise comparisons revealed significantly higher [tHb] values in the AF3 position than the AF4 position ( $p = 0.001$ ). Pairwise comparisons also revealed higher [tHb] values in the congruent block than the neutral blocks ( $p < 0.001$ ) and the incongruent blocks ( $p = 0.029$ ). Significant interaction effects were observed for Position x Rest,  $F(3,42) = 39.04, p < 0.001, \eta_p^2 = 0.74$ , Position x Block,  $F(2.12,29.66) = 62.25, p < 0.001, \eta_p^2 = 0.82$  and Rest x Block,  $F(1.20,16.78) = 15.50, p = 0.001, \eta_p^2 = 0.53$ .

For [O<sub>2</sub>Hb] values significant main effects were observed for Block,  $F(1.27,17.77) = 60.35, p < 0.001, \eta_p^2 = 0.81$ . Pairwise comparisons revealed significantly higher [O<sub>2</sub>Hb] values in the congruent blocks than the neutral ( $p < 0.001$ ) or the incongruent blocks ( $p < 0.001$ ) and significantly higher [O<sub>2</sub>Hb] values in the incongruent blocks than the neutral blocks ( $p < 0.001$ ). No significant main effects were observed for Position,  $F(3, 42) = 1.23, p = 0.31, \eta_p^2 = 0.08$  or Rest,  $F(1,14) = 1.27, p = 0.28, \eta_p^2 = 0.08$ . Significant interaction effects were revealed for Position x Rest,  $F(3,42) = 58.74, p < 0.001, \eta_p^2 = 0.81$ , Position x Block,  $F(1.38,19.36), = 83.81, p < 0.001, \eta_p^2 = 0.86$  and Rest x Block,  $F(1.31,18.39) = 68.25, p < 0.001, \eta_p^2 = 0.83$ .

Examination of [HHb] values revealed a significant main effect for Position,  $F(1.36,19.09) = 48.69, p < 0.001, \eta_p^2 = 0.78$  and Block,  $F(1,14) = 36.99, p < 0.001, \eta_p^2 = 0.73$ . Pairwise comparisons revealed that [HHb] values were higher in the AF4 position than the AF3 position ( $p < 0.001$ ), the Fp1 position ( $p < 0.001$ ) and the Fp2 position ( $p < 0.001$ ). [HHb] values were also higher in the Fp1 position than the Fp2 position ( $p = 0.007$ ). Pairwise comparisons of block effects revealed significantly higher [HHb] values in the congruent blocks than the neutral ( $p < 0.001$ ) and incongruent ( $p < 0.001$ ) blocks. No significant main effects were observed for Rest,  $F(1,14) = 0.78, p < 0.39, \eta_p^2 = 0.05$ . Significant interaction effects were revealed for Position x Rest,  $F(1.40,19.53) = 47.07, p < 0.001, \eta_p^2 = 0.77$ , Position x Block,  $F(1.13,15.78) = 54.68, p < 0.001, \eta_p^2 = 0.80$  and Rest x Block,  $F(1,14) = 42.99, p < 0.001, \eta_p^2 = 0.75$ .

#### *Position x Rest Interaction*

Paired t-tests revealed that [tHb] values were higher during exercise than at rest in the AF3 position,  $t = 4.12, p = 0.001$ , the Fp1 position,  $t = -4.45, p = 0.001$  and the Fp2 position,  $t = -2.20, p = 0.045$ . In the Af4 position [tHb] values were higher at rest than during exercise,  $t = 0.94, p < 0.001$ . Values for [O<sub>2</sub>Hb] were significantly higher during exercise than at rest in the AF3 position,  $t = -3.27, p = 0.006$  and the Fp1 position,  $t = -4.40, p = 0.001$ ). In the AF4 positions [O<sub>2</sub>Hb] values were significantly higher at rest than during exercise,  $t = 9.35, p < 0.001$ . Values for [HHb] were significantly higher during exercise than at rest in the AF3,  $t = -3.08, p = 0.008$ , AF4,  $t = -5.79, p < 0.001$  and Fp2,  $t = -2.30, p = 0.037$  positions. In the Fp1 position [HHb] values were higher at rest than during exercise,  $t = 7.32, p < 0.001$ .

### *Position x Block Interaction*

Separate repeated measures ANOVAs revealed that in the AF4 position [tHb] values were significantly higher in the congruent blocks than the neutral ( $p < 0.001$ ) and incongruent ( $p < 0.001$ ) blocks. [tHb] values were also higher in the neutral blocks than the incongruent blocks ( $p < 0.001$ ). In the Fp1 position [tHb] values were significantly higher in the incongruent blocks than the congruent ( $p = 0.001$ ) and neutral blocks ( $p = 0.001$ ). No significant differences in [tHb] values were observed between blocks in the AF3 or Fp2 positions ( $p > 0.05$ ). In the AF4 position [O<sub>2</sub>Hb] values were significantly higher in the congruent blocks than the neutral blocks ( $p < 0.001$ ) and incongruent blocks ( $p < 0.001$ ). [O<sub>2</sub>Hb] values were also significantly higher in the neutral blocks than the incongruent blocks ( $p = 0.004$ ). In the Fp1 position [O<sub>2</sub>Hb] values were significantly higher in the incongruent blocks than the congruent ( $p = 0.001$ ) and neutral blocks ( $p = 0.001$ ). No significant differences in [O<sub>2</sub>Hb] values were observed between blocks in the AF3 and Fp2 positions ( $p > 0.05$ ). In the AF4 position [HHb] values were significantly higher in the neutral blocks than the congruent blocks ( $p > 0.05$ ). [HHb] values were also significantly higher in the incongruent blocks than the congruent ( $p < 0.001$ ) and neutral ( $p = 0.013$ ) blocks. In the Fp1 position [HHb] values were significantly higher in the congruent blocks than the neutral ( $p < 0.001$ ) and incongruent ( $p < 0.001$ ) blocks. In the AF3 and Fp2 positions there were no significant differences between blocks ( $p > 0.05$ ).

### *Rest x Block Interaction*

Paired t-tests revealed that [tHb] values were higher during exercise than at rest in the neutral blocks  $t = -2.81, p = 0.014$  and the incongruent blocks  $t = -9.60, p < 0.001$ . There were no significant differences between values at rest and during exercise in the congruent blocks  $t = 1.19, p = 0.26$ . [O<sub>2</sub>Hb] values were significantly higher at rest than during exercise in the congruent block  $t = 5.51, p < 0.001$ . [O<sub>2</sub>Hb] values were significantly higher during exercise than at rest in the neutral  $t = -3.61, p = 0.003$  and incongruent  $t = -3.44, p = 0.004$  blocks. In the congruent blocks [HHb] values were significantly higher at rest than during exercise  $t = 5.37, p < 0.001$ . [HHb] values were significantly higher during exercise than at rest in the neutral  $t = -4.81, p < 0.001$  and incongruent  $t = 4.70, p < 0.001$  blocks.

### *Correlations with Stroop Response Time (resting trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.08, p = 0.77$ , [O<sub>2</sub>Hb],  $r = -0.13, p =$

0.66, or [HHb],  $r = -0.02$ ,  $p = 0.96$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.44$ ,  $p = 0.10$ , [O<sub>2</sub>Hb],  $r = -0.44$ ,  $p = 0.10$ , or [HHb],  $r = -0.41$ ,  $p = 0.13$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.26$ ,  $p = 0.34$ , [O<sub>2</sub>Hb],  $r = -0.25$ ,  $p = 0.37$ , or [HHb],  $r = 0.33$ ,  $p = 0.33$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = 0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = 0.08$ ,  $p = 0.78$ , or [HHb],  $r = 0.07$ ,  $p = 0.80$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.15$ ,  $p = 0.60$ , [O<sub>2</sub>Hb],  $r = -0.01$ ,  $p = 0.98$ , or [HHb],  $r = -0.34$ ,  $p = 0.22$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.08$ ,  $p = 0.77$ , [O<sub>2</sub>Hb],  $r = 0.08$ ,  $p = 0.79$ , or [HHb],  $r = -0.32$ ,  $p = 0.25$ .

In the Fp1 position significant negative correlations were found between [tHb], [O<sub>2</sub>Hb] and [HHb] in neutral and incongruent blocks and significant correlations were found between [tHb] and [O<sub>2</sub>Hb] in congruent blocks (see Table 3).

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.10$ ,  $p = 0.74$ , [O<sub>2</sub>Hb],  $r = -0.09$ ,  $p = 0.74$ , or [HHb],  $r = -0.10$ ,  $p = 0.73$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.07$ ,  $p = 0.80$ , [O<sub>2</sub>Hb],  $r = -0.06$ ,  $p = 0.82$ , or [HHb],  $r = -0.09$ ,  $p = 0.76$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.24$ ,  $p = 0.39$ , [O<sub>2</sub>Hb],  $r = -0.22$ ,  $p = 0.42$ , or [HHb],  $r = -0.26$ ,  $p = 0.34$ .

Table 3: Correlations between absolute values and Stroop response time in the Fp1 position during resting trials

STROOP trial	Oxygenation variable	Absolute value		Response time		Correlation (Pearson's $r$ )	Significance ( $p$ value)
		Mean	$SD$	Mean	$SD$		
Congruent	[tHb]	61.71	20.55	0.52	0.05	-0.56	0.031
	[O <sub>2</sub> Hb]	39.71	14.01	0.52	0.05	-0.58	0.024
	[HHb]	22.00	7.17	0.52	0.05	-0.46	0.078
Neutral	[tHb]	61.76	20.38	0.56	0.05	-0.58	0.025
	[O <sub>2</sub> Hb]	39.70	13.84	0.56	0.05	-0.58	0.024
	[HHb]	22.06	7.14	0.56	0.05	-0.52	0.046
Incongruent	[tHb]	61.71	20.27	0.59	0.05	-0.67	0.006
	[O <sub>2</sub> Hb]	39.63	13.71	0.59	0.05	-0.65	0.008
	[HHb]	22.08	7.18	0.59	0.05	-0.65	0.009

*Correlations with Stroop Accuracy (resting trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.09$ ,  $p = 0.75$ , [O<sub>2</sub>Hb],  $r = -0.15$ ,  $p = 0.60$  or [HHb],  $r = -0.01$ ,  $p = 0.98$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.03$ ,  $p = 0.93$ , [O<sub>2</sub>Hb],  $r = -0.04$ ,  $p = 0.88$ , or [HHb],  $r = 0.13$ ,  $p = 0.64$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = -0.21$ ,  $p = 0.45$ , [O<sub>2</sub>Hb],  $r = -0.28$ ,  $p = 0.32$ , or [HHb],  $r = -0.10$ ,  $p = 0.74$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.08$ ,  $p = 0.98$  or [HHb],  $r = -0.03$ ,  $p = 0.91$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = -0.03$ ,  $p = 0.91$ , [O<sub>2</sub>Hb],  $r = -0.09$ ,  $p = 0.76$ , or [HHb],  $r = 0.56$ ,  $p = 0.84$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = -0.08$ ,  $p = 0.78$ , [O<sub>2</sub>Hb],  $r = -0.07$ ,  $p = 0.81$ , or [HHb],  $r = -0.09$ ,  $p = 0.75$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.21$ ,  $p = 0.45$ , [O<sub>2</sub>Hb],  $r = 0.20$ ,  $p = 0.48$ ,

or [HHb]  $r = 0.23$ ,  $p = 0.42$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = -0.33$ ,  $p = 0.23$ , [O<sub>2</sub>Hb],  $r = -0.38$ ,  $p = 0.16$ , or [HHb],  $r = -0.20$ ,  $p = 0.48$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.12$ ,  $p = 0.68$ , [O<sub>2</sub>Hb],  $r = 0.12$ ,  $p = 0.68$ , or [HHb],  $r = 0.11$ ,  $p = 0.70$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.27$ ,  $p = 0.33$ , [O<sub>2</sub>Hb],  $r = 0.27$ ,  $p = 0.34$ , or [HHb],  $r = 0.27$ ,  $p = 0.33$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.06$ ,  $p = 0.82$ , [O<sub>2</sub>Hb],  $r = 0.08$ ,  $p = 0.77$ , or [HHb],  $r = 0.03$ ,  $p = 0.92$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.20$ ,  $p = 0.48$ , [O<sub>2</sub>Hb],  $r = 0.18$ ,  $p = 0.52$ , or [HHb],  $r = 0.22$ ,  $p = 0.43$ .

#### *Correlations with Stroop Response Time (exercise trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.41$ ,  $p = 0.14$ , [O<sub>2</sub>Hb], and  $r = -0.45$ ,  $p = 0.09$ , or [HHb],  $r = -0.29$ ,  $p = 0.30$ . Significant negative correlations were observed in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.53$ ,  $p = 0.44$  and [O<sub>2</sub>Hb],  $r = -0.58$ ,  $p = 0.02$ . No significant correlations were observed in the neutral blocks between Stroop response time and [HHb],  $r = -0.37$ ,  $p = 0.18$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.30$ ,  $p = 0.29$ , [O<sub>2</sub>Hb],  $r = -0.35$ ,  $p = 0.20$ , or [HHb],  $r = -0.16$ ,  $p = 0.56$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.24$ ,  $p = 0.40$ , [O<sub>2</sub>Hb],  $r = -0.17$ ,  $p = 0.54$ , or [HHb],  $r = -0.30$ ,  $p = 0.28$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.31$ ,  $p = 0.27$ , [O<sub>2</sub>Hb],  $r = -0.25$ ,  $p = 0.37$ , or [HHb],  $r = -0.38$ ,  $p = 0.16$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.11$ ,  $p = 0.69$ , [O<sub>2</sub>Hb],  $r = -0.03$ ,  $p = 0.93$ , or [HHb],  $r = -0.22$ ,  $p = 0.43$ .

In the Fp1 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.32$ ,  $p = 0.24$ , [O<sub>2</sub>Hb],  $r = -0.34$ ,  $p = 0.22$ , or [HHb],  $r = -0.26$ ,  $p = 0.35$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.31$ ,  $p = 0.54$ ,

[O<sub>2</sub>Hb],  $r = -0.06$ ,  $p = 0.83$ , or [HHb],  $r = -0.30$ ,  $p = 0.27$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.37$ ,  $p = 0.18$ , [O<sub>2</sub>Hb],  $r = -0.31$ ,  $p = 0.26$ , or [HHb],  $r = -0.44$ ,  $p = 0.11$ .

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.02$ ,  $p = 0.93$ , or [HHb],  $r = -0.006$ ,  $p = 0.98$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.01$ ,  $p = 0.99$ , [O<sub>2</sub>Hb],  $r = -0.01$ ,  $p = 0.99$ , or [HHb],  $r = -0.01$ ,  $p = 0.98$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.12$ ,  $p = 0.67$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.68$ , or [HHb],  $r = -0.12$ ,  $p = 0.67$ .

#### *Correlations with Stroop Accuracy (exercise trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.08$ ,  $p = 0.78$ , [O<sub>2</sub>Hb],  $r = 0.05$ ,  $p = 0.86$  or [HHb],  $r = 0.13$ ,  $p = 0.65$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = -0.44$ ,  $p = 0.11$ , [O<sub>2</sub>Hb],  $r = -0.48$ ,  $p = 0.07$ , or [HHb],  $r = -0.31$ ,  $p = 0.26$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = -0.16$ ,  $p = 0.57$ , [O<sub>2</sub>Hb],  $r = -0.20$ ,  $p = 0.47$ , or [HHb],  $r = -0.56$ ,  $p = 0.84$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.16$ ,  $p = 0.57$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.68$  or [HHb],  $r = 0.20$ ,  $p = -0.47$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = -0.35$ ,  $p = 0.20$ , [O<sub>2</sub>Hb],  $r = -0.43$ ,  $p = 0.11$ , or [HHb],  $r = -0.20$ ,  $p = 0.47$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.19$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.25$ ,  $p = 0.93$ , or [HHb],  $r = 0.08$ ,  $p = 0.77$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.74$ ,  $p = 0.79$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.67$ , or [HHb],  $r = 0.02$ ,  $p = 0.96$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = -0.30$ ,  $p = 0.92$ , [O<sub>2</sub>Hb],  $r = -0.06$ ,  $p = 0.83$ , or [HHb],  $r = 0.03$ ,  $p = 0.48$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.03$ ,  $p = 0.93$ , [O<sub>2</sub>Hb],  $r = -0.07$ ,  $p = 0.81$ , or [HHb]  $r = 0.06$ ,  $p = 0.85$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.05$ ,  $p = 0.87$ , [O<sub>2</sub>Hb],  $r = -0.09$ ,  $p = 0.74$ , or [HHb],  $r = 0.04$ ,  $p = 0.89$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = -0.10$ ,  $p = 0.73$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.68$ , or [HHb],  $r = -0.06$ ,  $p = 0.84$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.10$ ,  $p = 0.73$ , [O<sub>2</sub>Hb],  $r = 0.07$ ,  $p = 0.81$ , or [HHb],  $r = 0.15$ ,  $p = 0.58$ .

#### 4.4.3 Baseline NIRS Data

Examination of [tHb] values using a repeated measures ANOVA revealed that there were no significant main effects for Position,  $F(3,42) = 1.33$ ,  $p = 0.28$ ,  $\eta_p^2 = 0.09$ , Rest,  $F(1,14) = 1.50$ ,  $p = 0.24$ ,  $\eta_p^2 = 0.10$ , or Block,  $F(1.16,16.17) = 1.16$ ,  $p < 0.11$ ,  $\eta_p^2 = 0.17$ . There were also no significant interaction effects for Position x Rest,  $F(3,42) = 1.26$ ,  $p < 0.30$ ,  $\eta_p^2 = 0.08$ , Position x Block,  $F(1.98,27.69) = 0.63$ ,  $p < 0.54$ ,  $\eta_p^2 = 0.04$  or Rest x Block,  $F(1.29,18.02) = 0.43$ ,  $p = 0.46$ ,  $\eta_p^2 = 0.05$ .

For [O<sub>2</sub>Hb] values there were no significant main effects for Position,  $F(3,42) = 1.73$ ,  $p = 0.18$ ,  $\eta_p^2 = 0.11$ , Rest,  $F(1,14) = 0.20$ ,  $p = 0.66$ ,  $\eta_p^2 = 0.01$ , or Block,  $F(1.16,16.20) = 1.88$ ,  $p < 0.19$ ,  $\eta_p^2 = 0.12$ . There were also no significant interaction effects for Position x Rest,  $F(2.32,32.48) = 1.88$ ,  $p < 0.16$ ,  $\eta_p^2 = 0.12$ , Position x Block,  $F(1.61,22.55) = 0.37$ ,  $p < 0.65$ ,  $\eta_p^2 = 0.03$  or Rest x Block,  $F(1.21,16.87) = 0.89$ ,  $p = 0.38$ ,  $\eta_p^2 = 0.06$ .

Examination of [HHb] values revealed a significant main effect for Rest,  $F(1,14) = 26.71$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.66$ . There were no significant main effects for Position,  $F(1.89,26.39) = 1.45$ ,  $p = 0.25$ ,  $\eta_p^2 = 0.09$  or Block,  $F(3,42) = 0.92$ ,  $p = 0.41$ ,  $\eta_p^2 = 0.06$ . Pairwise comparisons revealed that [HHb] values were significantly higher during exercise than at rest ( $p < 0.001$ ). There were no significant interaction effects for Position x Rest,  $F(3,42) = 0.29$ ,  $p < 0.84$ ,  $\eta_p^2 = 0.02$ , Position x Block,  $F(2.87,40.21) = 0.87$ ,  $p < 0.46$ ,  $\eta_p^2 = 0.06$  or Rest x Block,  $F(1.40,19.53) = 0.70$ ,  $p = 0.46$ ,  $\eta_p^2 = 0.05$ .

#### *Correlations with Stroop Response Time (resting trials)*

In the AF4 position there was a significant correlation between Stroop response time and [O<sub>2</sub>Hb] in the congruent, neutral and incongruent blocks (see Table 4). There were no significant correlations during the congruent blocks between Stroop response time and values for [tHb] (see Table 4), however, in the neutral and incongruent blocks there were significant correlations between Stroop response time and [tHb] (see Table 4). There were

no significant correlations between Stroop response time and [HHb] in the congruent,  $r = -0.41, p = 0.14$ , neutral,  $r = 0.08, p = 0.79$  or incongruent,  $r = 0.13, p = 0.63$  blocks.

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.20, p = 0.49$ , [O<sub>2</sub>Hb],  $r = -0.02, p = 0.94$ , or [HHb],  $r = -0.50, p = 0.06$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.05, p = 0.87$ , [O<sub>2</sub>Hb],  $r = 0.21, p = 0.46$ , or [HHb],  $r = -0.42, p = 0.12$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.12, p = 0.66$ , [O<sub>2</sub>Hb]  $r = 0.18, p = 0.52$ , or [HHb],  $r = -0.50, p = 0.06$ .

In the Fp1 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = 0.31, p = 0.27$ , [O<sub>2</sub>Hb],  $r = 0.21, p = 0.46$ , or [HHb]  $r = 0.19, p = 0.50$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb]  $r = 0.01, p = 0.98$ , [O<sub>2</sub>Hb],  $r = -0.12, p = 0.67$ , or [HHb],  $r = 0.26, p = 0.34$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = 0.27, p = 0.33$ , [O<sub>2</sub>Hb],  $r = -0.03, p = 0.92$ , or [HHb],  $r = 0.39, p = 0.15$ .

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = 0.12, p = 0.66$ , [O<sub>2</sub>Hb],  $r = 0.02, p = 0.94$ , or [HHb],  $r = 0.21, p = 0.44$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = 0.03, p = 0.90$ , [O<sub>2</sub>Hb],  $r = -0.03, p = 0.93$ , or [HHb],  $r = 0.14, p = 0.63$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.09, p = 0.76$ , [O<sub>2</sub>Hb],  $r = -0.16, p = 0.57$ , or [HHb],  $r = 0.13, p = 0.64$ .

Table 4: Correlations of [tHb] and [O<sub>2</sub>Hb] values with Stroop response time using the Baseline and Zero methods of analysis during resting trials.

STROOP trial	Chromophore	Baseline		Zero	
		Mean ( $\pm$ SD)	Correlation (Pearson's $r$ )	Mean ( $\pm$ SD)	Correlation (Pearson's $r$ )
Congruent	[tHb]	0.31 (0.74)	0.44	-0.02 (0.49)	0.28
	[O <sub>2</sub> Hb]	0.44 (0.80)	0.57*	0.06 (0.47)	0.27
Neutral	[tHb]	0.50 (0.83)	0.64*	0.18 (0.39)	-0.24
	[O <sub>2</sub> Hb]	0.58 (0.75)	0.67**	0.17 (0.41)	-0.33
Incongruent	[tHb]	0.59 (0.72)	0.54*	0.09 (0.47)	0.52*
	[O <sub>2</sub> Hb]	0.71 (0.59)	0.58*	0.20 (0.52)	0.59*

Note: NIRS values are expressed in arbitrary units (A.U.) and mean  $\pm$  SD. \* =  $p < 0.05$ , \*\* =  $p < 0.01$

*Correlations with Stroop Accuracy (resting trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.002$ ,  $p = 0.96$ , [O<sub>2</sub>Hb],  $r = 0.14$ ,  $p = 0.63$  or [HHb],  $r = -0.34$ ,  $p = 0.22$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.08$ ,  $p = 0.78$ , [O<sub>2</sub>Hb],  $r = 0.16$ ,  $p = 0.58$ , or [HHb],  $r = -0.16$ ,  $p = 0.57$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.15$ ,  $p = 0.61$ , [O<sub>2</sub>Hb],  $r = 0.20$ ,  $p = 0.47$ , or [HHb],  $r = -0.04$ ,  $p = 0.88$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.08$ ,  $p = 0.98$  or [HHb],  $r = -0.03$ ,  $p = 0.91$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.24$ ,  $p = 0.38$ , [O<sub>2</sub>Hb],  $r = 0.26$ ,  $p = 0.35$ , or [HHb],  $r = 0.04$ ,  $p = 0.89$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.17$ ,  $p = 0.55$ , [O<sub>2</sub>Hb],  $r = 0.26$ ,  $p = 0.35$ , or [HHb],  $r = -0.15$ ,  $p = 0.60$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.11$ ,  $p = 0.71$ , [O<sub>2</sub>Hb],  $r = 0.06$ ,  $p = 0.83$ , or [HHb],  $r = -0.35$ ,  $p = 0.20$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.17$ ,  $p = 0.54$ , [O<sub>2</sub>Hb],  $r = -0.05$ ,  $p = 0.87$ ,

or [HHb],  $r = 0.45$ ,  $p = 0.09$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.16$ ,  $p = 0.58$ , [O<sub>2</sub>Hb],  $r = -0.25$ ,  $p = 0.37$ , or [HHb],  $r = 0.12$ ,  $p = 0.68$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.18$ ,  $p = 0.53$ , [O<sub>2</sub>Hb],  $r = -0.03$ ,  $p = 0.91$ , or [HHb],  $r = -0.32$ ,  $p = 0.24$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.22$ ,  $p = 0.43$ , [O<sub>2</sub>Hb],  $r = 0.04$ ,  $p = 0.90$ , or [HHb],  $r = 0.51$ ,  $p = 0.05$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.13$ ,  $p = 0.66$ , [O<sub>2</sub>Hb],  $r = 0.04$ ,  $p = 0.90$ , or [HHb],  $r = 0.21$ ,  $p = 0.45$ .

*Correlations with Stroop Response Time (exercise trials)* In the AF4 position there was a significant correlation between Stroop response time and both [tHb] and [O<sub>2</sub>Hb] in the congruent and neutral blocks (see Table 5). There were no significant correlations in the incongruent blocks between Stroop response time and [tHb],  $r = -0.25$ ,  $p = 0.36$ , or [O<sub>2</sub>Hb],  $r = -0.13$ ,  $p = 0.65$ . There were no significant correlations between Stroop response time and [HHb] in the congruent,  $r = 0.14$ ,  $p = 0.63$ , neutral,  $r = -0.17$ ,  $p = 0.56$  or incongruent,  $r = -0.23$ ,  $p = 0.42$  blocks.

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = 0.06$ ,  $p = 0.83$ , [O<sub>2</sub>Hb],  $r = 0.10$ ,  $p = 0.72$ , or [HHb],  $r = -0.08$ ,  $p = 0.77$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = 0.07$ ,  $p = 0.80$ , [O<sub>2</sub>Hb],  $r = 0.17$ ,  $p = 0.56$ , or [HHb],  $r = -0.31$ ,  $p = 0.26$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = 0.06$ ,  $p = 0.84$ , [O<sub>2</sub>Hb],  $r = 0.14$ ,  $p = 0.61$ , or [HHb],  $r = -0.35$ ,  $p = 0.20$ .

In the Fp1 position significant negative correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.55$ ,  $p = 0.03$ , and [O<sub>2</sub>Hb],  $r = -0.59$ ,  $p = 0.02$ . There were no significant correlations between Stroop response time and values for [HHb],  $r = 0.44$ ,  $p = 0.10$  in the congruent blocks. There were no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.47$ ,  $p = 0.08$ , [O<sub>2</sub>Hb],  $r = -0.51$ ,  $p = 0.05$ , or [HHb],  $r = 0.23$ ,  $p = 0.42$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.39$ ,  $p = 0.15$ , [O<sub>2</sub>Hb],  $r = -0.45$ ,  $p = 0.09$ , or [HHb],  $r = 0.42$ ,  $p = 0.12$ .

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.29$ ,  $p = 0.30$ , [O<sub>2</sub>Hb],  $r = -0.34$ ,  $p = 0.22$ , or [HHb],  $r = 0.19$ ,  $p = 0.50$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.22$ ,  $p = 0.44$ , [O<sub>2</sub>Hb],  $r = -0.21$ ,  $p = 0.44$ , or [HHb],  $r = -0.08$ ,  $p = 0.77$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.21$ ,  $p = 0.46$ , [O<sub>2</sub>Hb],  $r = -0.20$ ,  $p = 0.47$ , or [HHb],  $r = -0.06$ ,  $p = 0.84$ .

Table 5: The correlations of [tHb] and [O<sub>2</sub>Hb] values with Stroop response time using the baseline and zero methods of analysis during exercise trials.

STROOP trial	Oxygenation variable	Baseline		Zero	
		Mean ( $\pm$ SD)	Correlation (Pearson's $r$ )	Mean ( $\pm$ SD)	Correlation (Pearson's $r$ )
Congruent	[tHb]	0.47 (1.03)	-0.54*	0.90 (0.44)	-0.56*
	[O <sub>2</sub> Hb]	0.13 (1.06)	0.56*	-0.62 (0.50)	-0.48
Neutral	[tHb]	0.67 (1.08)	-0.65**	0.06 (0.29)	0.16
	[O <sub>2</sub> Hb]	0.29 (1.16)	-0.56*	-0.01 (0.35)	0.18
Incongruent	[tHb]	0.27 (1.09)	-0.25	0.46 (0.89)	0.54*
	[O <sub>2</sub> Hb]	-0.11 (1.41)	-0.13	0.34 (0.89)	-0.49

Note: NIRS values are expressed in arbitrary units (A.U.) and mean  $\pm$  SD. \* =  $p < 0.05$ , \*\* =  $p < 0.01$

#### *Correlations with Stroop Accuracy (exercise trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.07$ ,  $p = 0.81$ , [O<sub>2</sub>Hb],  $r = -0.02$ ,  $p = 0.96$  or [HHb],  $r = -0.17$ ,  $p = 0.55$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = -0.35$ ,  $p = 0.20$ , [O<sub>2</sub>Hb],  $r = -0.36$ ,  $p = 0.19$ , or [HHb],  $r = 0.08$ ,  $p = 0.77$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.14$ ,  $p = 0.61$ , [O<sub>2</sub>Hb],  $r = 0.10$ ,  $p = 0.72$ , or [HHb],  $r = 0.03$ ,  $p = 0.91$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.03$ ,  $p = 0.93$ , [O<sub>2</sub>Hb],  $r = -0.03$ ,  $p = 0.93$  or [HHb],  $r = -0.01$ ,  $p = 0.96$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = -0.07$ ,  $p = 0.80$ , [O<sub>2</sub>Hb],  $r = -0.02$ ,  $p = 0.94$ , or [HHb],  $r = -0.21$ ,  $p = 0.45$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.02$ ,  $p = 0.96$ , [O<sub>2</sub>Hb],  $r = -0.02$ ,  $p = 0.94$ , or [HHb],  $r = 0.15$ ,  $p = 0.59$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.18$ ,  $p = 0.52$ , [O<sub>2</sub>Hb],  $r = -0.17$ ,  $p = 0.54$ , or [HHb],  $r = -0.06$ ,  $p = 0.83$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = -0.15$ ,  $p = 0.59$ , [O<sub>2</sub>Hb],  $r = -0.15$ ,  $p = 0.59$ , or [HHb],  $r = 0.33$ ,  $p = 0.23$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.36$ ,  $p = 0.19$ , [O<sub>2</sub>Hb],  $r = -0.34$ ,  $p = 0.21$ , or [HHb],  $r = -0.11$ ,  $p = 0.70$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.07$ ,  $p = 0.82$ , or [HHb],  $r = 0.21$ ,  $p = 0.45$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.20$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.09$ ,  $p = 0.75$ , or [HHb],  $r = 0.35$ ,  $p = 0.20$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.03$ ,  $p = 0.92$ , [O<sub>2</sub>Hb],  $r = -0.11$ ,  $p = 0.71$ , or [HHb],  $r = 0.41$ ,  $p = 0.13$ .

#### 4.4.4 Zero NIRS Data

Examination of [tHb] values using a repeated measures ANOVA revealed that there were no significant main effects for Position,  $F(1.79, 25.09) = 0.82$ ,  $p = 0.44$ ,  $\eta_p^2 = 0.06$ , Rest,  $F(1, 14) = 0.89$ ,  $p = 0.36$ ,  $\eta_p^2 = 0.06$ , or Block,  $F(2, 28) = 0.05$ ,  $p < 0.95$ ,  $\eta_p^2 = 0.004$ . There were also no significant interaction effects for Position x Rest,  $F(1.98, 27.75) = 1.36$ ,  $p < 0.27$ ,  $\eta_p^2 = 0.09$ , Position x Block,  $F(3.14, 43.95) = 0.94$ ,  $p < 0.43$ ,  $\eta_p^2 = 0.06$  or Rest x Block,  $F(2, 28) = 1.50$ ,  $p = 0.24$ ,  $\eta_p^2 = 0.10$ .

For [O<sub>2</sub>Hb] values there were no significant main effects for Position,  $F(3, 42) = 0.12$ ,  $p = 0.95$ ,  $\eta_p^2 = 0.01$ , Rest,  $F(1, 14) = 0.13$ ,  $p = 0.72$ ,  $\eta_p^2 = 0.01$ , or Block,  $F(2, 28) = 0.01$ ,  $p < 0.99$ ,  $\eta_p^2 = 0.001$ . There were also no significant interaction effects for Position x Rest,  $F$

(3,42) = 1.12,  $p < 0.35$ ,  $\eta_p^2 = 0.07$ , Position x Block,  $F(6,84) = 1.20$ ,  $p < 0.32$ ,  $\eta_p^2 = 0.08$  or Rest x Block,  $F(2,28) = 1.74$ ,  $p = 0.20$ ,  $\eta_p^2 = 0.11$ .

Examination of [HHb] values revealed that there was a significant main effect for Rest,  $F(1,14) = 9.83$ ,  $p = 0.007$ ,  $\eta_p^2 = 0.41$ . There were no significant main effects for Position,  $F(1.49,20.86) = 1.17$ ,  $p = 0.32$ ,  $\eta_p^2 = 0.08$ , or Block,  $F(1.99,27.82) = 0.20$ ,  $p = 0.82$ ,  $\eta_p^2 = 0.01$ . Pairwise comparisons revealed that [HHb] values were significantly higher during exercise than at rest ( $p = 0.007$ ). There were no significant interaction effects for Position x Rest,  $F(1.21,16.99) = 0.89$ ,  $p < 0.38$ ,  $\eta_p^2 = 0.06$ , Position x Block,  $F(2.30,32.13) = 0.55$ ,  $p < 0.61$ ,  $\eta_p^2 = 0.04$  or Rest x Block,  $F(1.22,17.07) = 0.05$ ,  $p = 0.87$ ,  $\eta_p^2 = 0.004$ .

#### *Correlations with Stroop Response Time (resting trials)*

In the AF4 position there were no significant correlations between Stroop response time and [tHb] or [O<sub>2</sub>Hb] values in the congruent or neutral blocks (see Table 5). In the incongruent blocks there were significant correlations between Stroop response time and values for [tHb] and [O<sub>2</sub>Hb] (see Table 5). There were no significant correlations between Stroop response time and [HHb] in the congruent,  $r = 0.05$ ,  $p = 0.85$ , neutral,  $r = 0.24$ ,  $p = 0.40$  or incongruent,  $r = -0.27$ ,  $p = 0.33$  blocks.

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.33$ ,  $p = 0.24$ , [O<sub>2</sub>Hb],  $r = -0.24$ ,  $p = 0.40$ , or [HHb],  $r = -0.18$ ,  $p = 0.53$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.23$ ,  $p = 0.40$ , [O<sub>2</sub>Hb],  $r = -0.21$ ,  $p = 0.46$ , or [HHb],  $r = -0.10$ ,  $p = 0.73$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.11$ ,  $p = 0.70$ , [O<sub>2</sub>Hb],  $r = 0.12$ ,  $p = 0.67$ , or [HHb],  $r = -0.47$ ,  $p = 0.08$ .

In the Fp1 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = 0.12$ ,  $p = 0.66$ , [O<sub>2</sub>Hb],  $r = 0.10$ ,  $p = 0.72$ , or [HHb],  $r = 0.12$ ,  $p = 0.68$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.21$ ,  $p = 0.45$ , [O<sub>2</sub>Hb],  $r = -0.10$ ,  $p = 0.71$ , or [HHb],  $r = -0.33$ ,  $p = 0.24$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.11$ ,  $p = 0.70$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.67$ , or [HHb],  $r = 0.01$ ,  $p = 0.99$ .

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.10$ ,  $p = 0.73$ , [O<sub>2</sub>Hb],  $r = -$

0.06,  $p = 0.84$ , or [HHb],  $r = -0.17$ ,  $p = 0.56$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = 0.31$ ,  $p = 0.26$ , [O<sub>2</sub>Hb],  $r = 0.11$ ,  $p = 0.71$ , or [HHb],  $r = 0.26$ ,  $p = 0.34$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = 0.02$ ,  $p = 0.95$ , [O<sub>2</sub>Hb],  $r = -0.12$ ,  $p = 0.68$ , or [HHb],  $r = 0.16$ ,  $p = 0.58$ .

*Correlations with Stroop Accuracy (resting trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.13$ ,  $p = 0.64$ , [O<sub>2</sub>Hb],  $r = 0.003$ ,  $p = 0.99$  or [HHb],  $r = -0.35$ ,  $p = 0.20$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.33$ ,  $p = 0.23$ , [O<sub>2</sub>Hb],  $r = 0.43$ ,  $p = 0.11$ , or [HHb],  $r = -0.28$ ,  $p = 0.32$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = 0.49$ ,  $p = 0.07$ , [O<sub>2</sub>Hb],  $r = 0.50$ ,  $p = 0.06$ , or [HHb],  $r = -0.13$ ,  $p = 0.64$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.17$ ,  $p = 0.54$ , [O<sub>2</sub>Hb],  $r = -0.05$ ,  $p = 0.87$  or [HHb],  $r = -0.25$ ,  $p = 0.37$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.39$ ,  $p = 0.15$ , [O<sub>2</sub>Hb],  $r = 0.42$ ,  $p = 0.12$ , or [HHb],  $r = -0.05$ ,  $p = 0.86$ . In the incongruent blocks there was a significant correlation between Stroop accuracy and values for [O<sub>2</sub>Hb],  $r = 0.53$ ,  $p = 0.04$ . There were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.46$ ,  $p = 0.08$ , or [HHb],  $r = -0.02$ ,  $p = 0.94$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.16$ ,  $p = 0.57$ , [O<sub>2</sub>Hb],  $r = 0.14$ ,  $p = 0.61$ , or [HHb],  $r = 0.12$ ,  $p = 0.67$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.03$ ,  $p = 0.93$ , [O<sub>2</sub>Hb],  $r = 0.16$ ,  $p = 0.56$ , or [HHb],  $r = -0.29$ ,  $p = 0.29$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.26$ ,  $p = 0.34$ , [O<sub>2</sub>Hb],  $r = -0.26$ ,  $p = 0.35$ , or [HHb],  $r = -0.05$ ,  $p = 0.85$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.07$ ,  $p = 0.80$ , [O<sub>2</sub>Hb],  $r = 0.06$ ,  $p = 0.85$ , or [HHb],  $r = 0.08$ ,  $p = 0.79$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.06$ ,  $p = 0.82$ , [O<sub>2</sub>Hb],  $r = 0.03$ ,  $p = 0.91$ ,

or [HHb],  $r = 0.04$ ,  $p = 0.90$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = 0.01$ ,  $p = 0.96$ , [O<sub>2</sub>Hb],  $r = -0.02$ ,  $p = 0.93$ , or [HHb],  $r = 0.04$ ,  $p = 0.88$ .

*Correlations with Stroop Response Time (exercise trials)*

In the AF4 position there was a significant correlation between Stroop response time and [tHb] in the congruent and incongruent blocks (see Table 5). In the neutral blocks there were no significant correlations between Stroop response time and values for [tHb]. There were no significant correlations in the congruent, neutral and incongruent blocks between Stroop response time and values for [O<sub>2</sub>Hb] (see Table 5). There were no significant correlations between Stroop response time and values for [HHb] in the congruent,  $r = -0.03$ ,  $p = 0.91$ , neutral,  $r = 0.33$ ,  $p = 0.23$ , or incongruent,  $r = -0.29$ ,  $p = 0.30$  blocks.

In the AF3 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.08$ ,  $p = 0.77$ , [O<sub>2</sub>Hb],  $r = -0.07$ ,  $p = 0.81$ , or [HHb],  $r = -0.07$ ,  $p = 0.79$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = 0.29$ ,  $p = 0.30$ , [O<sub>2</sub>Hb],  $r = 0.21$ ,  $p = 0.46$ , or [HHb],  $r = 0.41$ ,  $p = 0.13$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.23$ ,  $p = 0.42$ , [O<sub>2</sub>Hb],  $r = -0.08$ ,  $p = 0.77$ , or [HHb],  $r = -0.38$ ,  $p = 0.17$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop response time and values for [tHb],  $r = 0.03$ ,  $p = 0.92$ , [O<sub>2</sub>Hb],  $r = -0.25$ ,  $p = 0.37$  or [HHb],  $r = 0.38$ ,  $p = 0.16$ . There were no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = 0.02$ ,  $p = 0.96$ , [O<sub>2</sub>Hb],  $r = -0.20$ ,  $p = 0.47$ , or [HHb],  $r = 0.34$ ,  $p = 0.21$ . In the incongruent blocks there were no significant correlations observed between Stroop response time and values for [tHb],  $r = -0.43$ ,  $p = 0.11$ , [O<sub>2</sub>Hb],  $r = -0.52$ ,  $p = 0.05$ , or [HHb],  $r = 0.18$ ,  $p = 0.53$ .

In the Fp2 position no significant correlations were found during the congruent blocks between Stroop response time and values for [tHb],  $r = -0.16$ ,  $p = 0.57$ , [O<sub>2</sub>Hb],  $r = -0.30$ ,  $p = 0.92$ , or [HHb],  $r = -0.19$ ,  $p = 0.50$ . There were also no significant correlations in the neutral blocks between Stroop response time and values for [tHb],  $r = -0.18$ ,  $p = 0.52$ , [O<sub>2</sub>Hb],  $r = -0.26$ ,  $p = 0.36$ , or [HHb],  $r = 0.002$ ,  $p = 0.99$ . In the incongruent blocks no significant correlations were observed between Stroop response time and values for [tHb],  $r = -0.06$ ,  $p = 0.84$ , [O<sub>2</sub>Hb],  $r = -0.16$ ,  $p = 0.57$ , or [HHb],  $r = 0.06$ ,  $p = 0.84$ .

### *Correlations with Stroop Accuracy (exercise trials)*

In the AF4 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.18$ ,  $p = 0.53$ , [O<sub>2</sub>Hb],  $r = -0.29$ ,  $p = 0.30$  or [HHb],  $r = 0.38$ ,  $p = 0.16$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.16$ ,  $p = 0.57$ , [O<sub>2</sub>Hb],  $r = 0.18$ ,  $p = 0.53$ , or [HHb],  $r = -0.08$ ,  $p = 0.78$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = -0.28$ ,  $p = 0.31$ , [O<sub>2</sub>Hb],  $r = -0.28$ ,  $p = 0.31$ , or [HHb],  $r = -0.02$ ,  $p = 0.95$ .

In the AF3 position no significant correlations were found during the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.32$ ,  $p = 0.24$ , [O<sub>2</sub>Hb],  $r = -0.33$ ,  $p = 0.23$  or [HHb],  $r = -0.05$ ,  $p = 0.86$ . In the neutral blocks no significant correlations were observed between Stroop accuracy and values for [tHb],  $r = 0.30$ ,  $p = 0.27$ , [O<sub>2</sub>Hb],  $r = 0.30$ ,  $p = 0.28$ , or [HHb],  $r = 0.10$ ,  $p = 0.71$ . No significant correlations were found in the incongruent blocks between Stroop accuracy and values for [tHb],  $r = -0.22$ ,  $p = 0.43$ , [O<sub>2</sub>Hb],  $r = -0.18$ ,  $p = 0.53$ , or [HHb],  $r = -0.13$ ,  $p = 0.65$ .

In the Fp1 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = -0.18$ ,  $p = 0.52$ , [O<sub>2</sub>Hb],  $r = -0.50$ ,  $p = 0.86$ , or [HHb],  $r = -0.25$ ,  $p = 0.38$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = -0.03$ ,  $p = 0.92$ , [O<sub>2</sub>Hb],  $r = -0.14$ ,  $p = 0.62$ , or [HHb],  $r = 0.16$ ,  $p = 0.57$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.06$ ,  $p = 0.82$ , [O<sub>2</sub>Hb],  $r = -0.39$ ,  $p = 0.16$ , or [HHb],  $r = 0.49$ ,  $p = 0.06$ .

In the Fp2 position there were no significant correlations in the congruent blocks between Stroop accuracy and values for [tHb],  $r = 0.02$ ,  $p = 0.94$ , [O<sub>2</sub>Hb],  $r = 0.35$ ,  $p = 0.20$ , or [HHb],  $r = -0.02$ ,  $p = 0.95$ . No significant correlations were observed in the neutral blocks between Stroop accuracy and values for [tHb],  $r = 0.13$ ,  $p = 0.64$ , [O<sub>2</sub>Hb],  $r = 0.35$ ,  $p = 0.21$ , or [HHb],  $r = -0.18$ ,  $p = 0.52$ . In the incongruent blocks there were no significant correlations between Stroop accuracy and values for [tHb],  $r = -0.20$ ,  $p = 0.47$ , [O<sub>2</sub>Hb],  $r = -0.16$ ,  $p = 0.58$ , or [HHb],  $r = 0.20$ ,  $p = 0.47$ .

## **4.5 Discussion**

This study aimed to establish the optimum techniques for processing NIRS data by comparing absolute and relative techniques at rest and during exercise. This study also

aimed to determine the optimum NIRS probe position to assess the haemodynamic responses to neural activation at rest and during exercise. The findings of this study indicate that relative analysis of NIRS data has the sensitivity to detect correlations between oxygenation variables and Stroop performance when processed using both the zero and baseline methods of processing and that these correlations are situated in the AF4 position. The baseline method has a greater ability to detect smaller changes such as those observed during congruent and neutral blocks. Therefore, the hypothesis that the use of an absolute zero would be the optimum method of processing the data must be rejected. Furthermore, the hypothesis that the strongest response to the Stroop test would be on the left side of the prefrontal cortex (Fp1/AF3) must also be rejected.

#### **4.5.1 NIRS probe positioning**

The association between the AF4 position, situated on the right side of the prefrontal cortex, and Stroop performance, whilst indicated in some studies (Millham et al, 2001; Vendrell, Junqué, Pujol, Jurado, Molet & Graffman, 1995), is in contrast to many previous studies which have indicated the left side of the prefrontal cortex as involved in the Stroop interference effect (e.g., Milham, Banich, Barard, 2003; Taylor, Kornblum, Lauber, Minoshima & Koeppel, 1997; Vanderhasselt, Raedt, Baeken, Leyman & D'haenen, 2006). The right side of the prefrontal cortex has a role in attention and response inhibition (Casey et al., 1997; Knight, Grabowecky & Scabini, 1995; Rubia, Smith, Brammer & Taylor, 2003) and thus the correlations observed in this study are likely to reflect this. These findings indicate that the AF4 position has a role in attention and may therefore be a suitable position to use to assess neurological responses to a dual task protocol.

#### **4.5.2 Absolute NIRS data**

Whilst the absolute values in this study did not reveal any positive correlations with Stroop response time or accuracy, negative correlations with oxygenation variables were observed in the Fp1 position in congruent, neutral and incongruent trials in the resting condition. The Fp1 position is situated on the left side of the prefrontal cortex and therefore changes here are more in line with previous studies (Milham et al., 2003; Taylor et al., 2006). However, a negative correlation suggests a decrease in blood flow and oxygenation in relation to Stroop performance rather than the increase that would be expected to occur in response to neurological activation. The absolute values were more sensitive than the relative values to changes in response to exercise and consequently these negative correlations may

simply reflect a reduction in blood flow to this region. This interpretation can be supported by the fact that the Fp1 position was the only one not to demonstrate a significant difference between resting and exercise values.

Changes in cerebral blood flow are influenced by exercise (Ogoh & Ainslie, 2009), as well as in response to neurological activation (Yanagisawa et al., 2010) and a hemispheric specific response has been observed with localised decreases in blood flow to regions contralateral to the activated areas (De Joux et al., 2017; Hoshi & Tamura, 1997), a fact which provides an explanation for the results presented in this chapter. The results of this study indicate that the validity of absolute [tHb] values for assessing changes in the calf observed by Stone et al. (2016) are likely to be due to the sensitivity of absolute values to changes in blood flow. For cognitive measurements the findings of this study indicate that whilst absolute values are more suitable than relative values for detecting changes between rest and exercise, unlike the relative methods they lack the sensitivity to determine changes in neurological activity.

#### **4.5.3 Relative NIRS data**

Whilst both the zero and baseline methods of data processing both produced correlations with Stroop response time, the baseline method produced correlations for more conditions and variables. By subtracting a 1000ms baseline value from the absolute values recorded by the NIRS software, correlations with Stroop response time were found with [O<sub>2</sub>Hb] across all resting Stroop trials and congruent and neutral trials during exercise and with [tHb] during resting neutral and incongruent trials and exercise congruent and neutral trials. More correlations with behavioural data were found using this method of processing than using the zero method, which only revealed significant correlations between Stroop response time and [O<sub>2</sub>Hb] during resting incongruent trials, and between Stroop response time and [tHb] during resting incongruent trials and exercise congruent and incongruent trials. It is generally considered that an increase in [O<sub>2</sub>Hb] is the strongest indicator of increases in regional neurological activation (Plichta et al., 2006; Schecklmann et al., 2008) and consequently it would seem that the baseline method of analysis produces more relevant results as it was able to detect activation in the easier congruent and neutral trials (Duncan-Johnson & Kopell, 1981, Milham et al., 2001).

#### **4.5.4 Limitations**

The primary limitation which must be considered before interpreting and applying the results is the positioning of the NIRS probes. This was achieved using the 10-20 electrode positioning system which involves manual measurements of the appropriate position based on percentages of head circumference and length (Jasper, 1958). This method has been established as an appropriate method for locating brain regions without conducting a detailed brain scan, however, brain anatomy differs between people (Schecklmann et al., 2008) and as such accuracy of anatomical locations cannot be guaranteed. Furthermore, this method uses manual assessments and therefore is subject to human error. The correlation with behavioural data was used to determine the optimum method of processing, however, the use of correlations with behavioural data does not always produce an accurate determination of neurological responses (Jaeggi et al., 2003) so this must be considered when interpreting the findings presented in this chapter. There are also a number of limitations of the use of NIRS which are discussed in detail in section 2.5.6. Furthermore, the use of a single exercise intensity limits the conclusions that can be drawn from the responses to exercise.

#### **4.6 Conclusion**

This study has demonstrated that relative values are more appropriate than absolute values for assessing neurological responses to a cognitive stimulus, particularly when analysed relative to a change from a 1000ms baseline, both at rest and during exercise. Values determined relative to a baseline possess more sensitivity to easier tasks and provide stronger correlations with [O<sub>2</sub>Hb] which is the chief molecule to be considered when examining neurological responses. The study has also indicated a role of the AF4 region in Stroop performance which suggests a potential role for this region in attention related activities such as a dual task paradigm.

This chapter has established the appropriate method of analysing NIRS data obtained and demonstrated that a single position NIRS probe is sensitive enough to determine changes in neurological activation. Whilst a role for the AF4 region has been indicated this should be confirmed against a more established method of neurological analysis. Subsequent chapters will examine the validity of measurements obtained by comparing results against those obtained using an electroencephalogram (EEG) and determine the within and between day reliability of the data obtained using this technique.

## **Chapter 5: The validity of the Artinis Portalite single position NIRS device for determining cerebral haemodynamic changes in responses to a cognitive stimulus**

### **5.1 Introduction**

In order to ascertain that the response to an experimental manipulation is truly a result of that manipulation and not due to other factors, we must first ascertain that the measurement technique we are using measures the response we are looking to examine. This phenomenon is described in research as validity testing (Jones & Gratton, 2014). In the previous chapter the Artinis Portalite NIRS device was examined in order to determine the optimal way to process the data obtained. It was established that the strongest relationship with behavioural performance occurred when calculating change from a 10 second baseline taken from directly before the start of the cognitive stimulus (in this case a Stroop colour-word test). Using correlations with behavioural data (Stroop response time) it was also established that Stroop induced neurological activation was lateralised to the right side of the prefrontal cortex.

Whilst correlations with behavioural data are the standard way to assess neurological responses as measured by NIRS (Schroeter et al., 2002), correlations with behavioural data within a specific neurological region are not necessarily evidence of the involvement of that region in completion of the task (Jaeggi et al., 2003). Criterion validity testing (Atkinson & Nevill, 2001) can be used to confirm that the correlations between NIRS and Stroop performance are evidence of neurological activation by comparing the data obtained using NIRS to data obtained using an established neuropsychological measurement technique such as EEG. This chapter will compare data collected using the Artinis Portalite NIRS device to data collected using an EEG to establish whether the correlations with behavioural data observed in the previous chapter are evidence of neurological activation.

#### **5.1.2 The Importance of validity measurements**

The validity of a research tool or technique is a cornerstone of empirical enquiry (Atkinson & Nevill, 1998) and is often discussed in combination with reliability, a factor which is

examined in the next chapter. One component of validity testing is internal validity, which includes how well a piece of equipment or instrumentation measures the phenomenon being investigated (Jones & Gratton, 2014). This thesis is focussed on validating the Artinis Portalite NIRS device for assessing cognitive responses and therefore is concentrating on an aspect of internal validity. One way of validating a technique or piece of equipment is to compare it to another technique which has already been established as a valid tool for detecting the change or response you are interested in. Validity can be said to be represented by the extent to which two techniques measure the same trait through different methods (Campbell & Fisk, 1959). Therefore, in order to establish the validity of the Artinis Portalite NIRS device for assessing neural activation in response to a stimulus it is useful to compare the device against an established method of assessing neural activation in response to a cognitive stimulus.

### **5.1.3 Comparison of NIRS to other neuroimaging techniques**

In neuropsychological research there are a number of functional imaging techniques which are used to assess responses to cognitive stimuli. In addition to NIRS and fNIRS devices the most common techniques used are BOLD fMRI and EEG. Similar to NIRS, BOLD fMRI relies on examining the changes in haemoglobin status within the blood to detect changes in brain activity (D'Esposito, Deouell & Gazzaley, 2003). BOLD fMRI relies on the paramagnetic properties of [HHb] to determine neural activation (Kim & Bandettini, 2012; Logothetis & Pfeuffer, 2004). Unlike NIRS fMRI scanners have excellent spatial resolution and can produce detailed images of changes in activation throughout the whole brain, but these scanners are high cost, need extensive training to operate, and require the subject to lie in a prone position and be completely stationary, resulting in a lack of ecological validity (Scarapicchia, Brown, Mayo & Gawryluk, 2017). A number of studies have demonstrated that changes in brain activation detected by fNIRS devices correlate well with BOLD fMRI findings (e.g., Alderliesten et al., 2014; Kleinschmidt et al., 2006) (see section 2.4.3 for an overview). Whilst a certain correlation with BOLD fMRI might be expected as they both rely on detecting changes in the haemodynamic response to a stimulus, NIRS devices have also been shown to produce measurements that correlate well with those obtained using EEG (Butti et al., 2006).

#### **5.1.4 EEG**

EEG relies on electrodes positioned on the scalp to detect electrical signals within the brain created by the transmission of nerve impulses and this technique has increased in prominence since the development of computers with a high storage capacity in the 1990s (Schomer, da Silva, Sutter, Caplan & Schomer, 2017). EEG is currently one of the most commonly used tools in psychological research (Butti et al., 2006). The EEG is a less restrictive neuroimaging technique than fMRI as the subject is able to maintain a seated position during the measurement. Whilst EEG recordings can be made with a high temporal resolution, they are still very sensitive to movement artefacts (Butti et al., 2006; Canning & Sheutz, 2013; Teplan, 2002) and thus also lack ecological validity. Some EEG systems have now been developed to be used during activity by applying robust movement filters (Thompson, Steffert, Ros, Leach & Gruzelier, 2008), but the effect of these filters on the quality of the data obtained has yet to be established. Despite the limitations to movement, the EEG has been determined to be highly accurate in determining localised neural activity (Arefian et al., 2012; Cook, O'Hara, Uijtdehaage, Mandelkern & Leuchter, 1998; Cuffin et al., 1991; Walczak, Radtke & Lewis, 1992) and therefore provides a well validated neuroimaging tool against which the findings obtained using the Artinis Portalite NIRS device can be compared.

#### **5.1.5 Determination of optimum positioning**

In the previous chapter correlations were found between performance data and Stroop performance in the AF4 position which corresponds to the right side of the dorsolateral prefrontal cortex (Brodmann's area 9/46). Whilst some studies have supported a role for this region in the Stroop interference effect (Vanderhasselt, De Raedt & Baeken, 2009; Vendrell et al., 1995), the majority of studies demonstrate left prefrontal lateralisation of the Stroop effect (e.g., Adleman et al., 2002; MacDonald, Cohen, Stenger & Carter, 2002; Stuss, Floden, Alexander, Levine & Katz, 2001; Yanagisawa et al., 2010). Therefore, it is important to determine whether this right lateralisation effect can be repeated and whether it is also detected by the EEG which would confirm the accuracy of the NIRS device for detecting neurological activation induced by a cognitive task.

### **5.1.6 Stroop**

As with the previous chapter the cognitive test used to induce a neurological response was the Stroop colour word test. The rationale underpinning the use of the Stroop colour word test is explained in section 4.1.2. In the previous chapter a neutral trial was included, in addition to the congruent and incongruent trials that are standard in a Stroop test, however, as neutral trials are rarely discussed in the literature this was excluded from the current study in order that comparisons could be made between the positioning data obtained in this study and that detailed in the literature. The Stroop colour-word task was chosen for this chapter due to the known activation of the prefrontal cortex elicited by the Stroop protocol (Liu, Banich, Jacobson & Tanabe, 2004) a region which is of interest in the monitoring of dual task effects (Leone et al., 2017). This task was also chosen due to the similarity of the cognitive demands of the Stroop test to those induced by dual task protocols (Hommel & Eglau, 2002).

### **5.1.7 Validity of the Artinis Portalite NIRS device**

A number of studies have utilised the single position Artinis Portalite NIRS device to measure cerebral oxygenation (e.g., Porcelli et al., 2010; Rupp et al., 2013; Smith & Billaut, 2010; Subudhi, Dimmen & Roach, 2007), however, as yet the only study to examine the validity of this device has been that of Stone et al. (2016) who confirmed the validity of the Artinis Portalite for assessing leg blood volume. One recent study has examined the sensitivity of the Artinis Portalite for assessing cerebral oxygenation responses to postural changes (Moi, Woltering, Collier, Maier, Meskers & van Wezel, 2019), however, this study did not compare the findings against any alternative methods and consequently was not able to establish validity. It is important therefore, that the validity of this device for cerebral measurements is confirmed.

## **5.2 Aims**

The aim of this study was to determine the validity of the Artinis Portalite NIRS device in determining the haemodynamic responses to neural activity. Neurological activation measured by the Artinis Portalite and an EEG system were compared to determine if activation was measured in the same regions of the prefrontal cortex. Furthermore, this chapter aimed to determine the optimum positioning for the a single position NIRS device

to determine activation in the prefrontal cortex. A Stroop colour word task which is known to activate the prefrontal cortex was used to determine optimum positioning. The experimental questions that were addressed in this chapter are:

1. Do the responses to neural activation recorded by the Artinis Portalite NIRS at rest device correlate with those recorded by an EEG?
2. Do the responses to neural activation recorded by the Artinis Portalite NIRS during exercise correlate with behavioural measures in the same manner as those recorded at rest?
3. Which region of the prefrontal cortex is activated in response to the Stroop colour word task?

It is hypothesised that neurological activation recorded by the Artinis Portalite will correlate well with activation detected by the EEG. Furthermore, based on the behavioural correlations observed in the previous chapter this activation is expected to be lateralised to the right side of the DLPFC.

## 5.3 Methods

### 5.3.1 Participants

Twenty-four participants (16 male, 8 female; mean age:  $24.54 \pm 6.34$  years; stature:  $1.70 \pm 0.08$  m; body mass  $71.0 \pm 11.77$  kg) were recruited to take part in this study using convenience sampling. Participants were identified from a sample of university students and staff using the following inclusion and exclusion criteria:

#### *Inclusion criteria*

- Male or female
- Aged 18-40 years

#### *Exclusion criteria*

- Colour blind
- Suffering from any physical illness that would preclude maximal exercise testing
- High blood pressure
- Suffering from any injury that would prevent cycling at maximal intensity

All participants volunteered to participate in the study and provided written informed consent (see appendix A) to take part following the provision of a participant information sheet (see appendix B). Participants were instructed to avoid exercise and caffeine for 24 hours prior to each testing session and to arrive at the laboratory at least 3 hours postprandial in a fully rested and hydrated state. Participants completed a physical readiness for activity (PAR-Q) questionnaire (see appendix C) and had a blood pressure measurement taken before the start of each testing session. Ethical approval was obtained from the University of Winchester ethics committee before the commencement of this study.

### **5.3.2 Sample size determination**

A sample size of 24 was determined to be sufficient to detect significant effects with power at the 0.80 level and an alpha of 0.05 as predicted by G\*power (Faul, Erdfelder, Lang & Buchner, 2007). The power determination was based on the results of Ambrosini & Valessi (2017). The sample size calculation was based on the effect size of  $d = 0.70$  detailed in the paper in relation to Stroop interference effects.

### **5.3.3 Experimental procedure**

Participants attended four testing sessions over a 2-4 week period with a minimum of 48 hours between sessions. All testing sessions were completed at the same time of day ( $\pm 2$  hours) and the same laboratory was used for all participants. Exercise tests were completed in a temperature controlled exercise laboratory and EEG tests were completed in a soundproof cubicle within a different laboratory. Participants completed a graded exercise test, two NIRS trials which consisted of four resting Stroop tests and four exercise Stroop tests while cycling at 90% GET, and one EEG session which consisted of four resting Stroop tests. The two NIRS trials and EEG trial were completed in a randomised counterbalanced order to exclude any familiarisation effects.

### **5.3.4 Graded exercise test (GXT)**

During the initial testing session participants completed a graded exercise test (GXT) using a ramp protocol which is described in detail in section 4.3.4. The test was completed on an electronically braked cycle ergometer (SRM Ergometer, Jülich, Germany) and breath by breath data was collected using an online gas analyser (Cortex Biophysik, Leipzig, Germany). Participants commenced the test by cycling for five minutes with resistance set at 0 watts

(W), which was used as a warm-up period. Following this five minute period the cycle ergometer increased the pedal resistance by 1 W every 3 seconds. Participants were instructed to maintain a cadence of ~75 revolutions per minute (rpm) and to continue cycling until they could no longer maintain this cadence. The test was concluded either by the participant determining they could no longer continue or by the researcher instructing the participant to stop due to cadence dropping below 70 rpm. Cadence, pedal resistance and duration of test were all displayed on a computer monitor placed on a table directly in front of the cycle ergometer. Heart rate during the test was recorded using a chest strap and watch (Polar Electro UK Ltd., Warwick, England).

### **5.3.5 Determination of Gas Exchange Threshold (GET)**

The gas exchange threshold (GET) was determined for each participant from a graph of the  $\text{VO}_2$  response using the modified V-slope method (Beaver, Wassermann & Whipp, 1986; Davis, 1985) and independently verified by two researchers. The rationale for the use of the GET to determine exercise intensity and a full explanation of the protocol can be found in section 4.3.5 Also see Figure 9 for a graphical representation of the method. Following determination of the GET a work rate equal to 90% GET was determined and this work rate was used as the exercise intensity during the exercise Stroop tests.

### **5.3.6 Stroop test protocol**

The Stroop test protocol consisted of one block of 36 congruent trials (e.g., the word red written in the colour red) followed by one block of 36 incongruent trials (e.g., the word red written in the colour blue) (see Figure 15). Each stimulus (word) was preceded by a 300 ms fixation cross, followed by presentation of the stimulus for 1200 ms and finally a 500 ms interval before the presentation of the next stimulus. These timings were based on the Stroop test protocol described by Milham et al. (2001). Each Stroop test consisted of 72 trials and took approximately 2.5 minutes to complete. Congruent trials were always presented before incongruent trials with a 2000 ms fixation cross between blocks. A wireless keyboard was used to collect responses to the Stroop protocol with colour stickers affixed to the keys (see Figure 11). The Stroop test was designed using the open-source software Psychopy (Peirce, 2007) and presented on a Lenovo Ideapad 500 laptop with a 17" screen. The laptop was positioned 170 cm in front of participants during all trials and screen brightness was kept at a constant level throughout all sessions.



Figure 15: An example of a congruent (left) and incongruent (right) trial and a fixation cross as presented to participants

### 5.3.7. NIRS Experimental trials

The same protocol was followed in both the NIRS experimental trials. The trial initiated with the participant remaining seated for five minutes whilst resting HR was measured and measurements were taken for positioning the two NIRS probes in either the AF3 and AF4 positions or the Fp1 and Fp2 positions based on the modified 10-20 electrode positioning system (Jasper, 1958) (see Figure 12). The four positions are all located on the forehead with the Fp1 and Fp2 positions located approximately above the middle of the eyebrow on the left and right side of the face respectively and the AF3 and AF4 positions located between this position and the hairline at the side of the face on the left and right sides respectively. Detailed measurements for locating the respective positions are outlined in appendix D.

NIRS probes were affixed to the head using bi-adhesive tape to minimise movement artefacts created by the probe de-coupling from the head (Scheeren, Schober & Schwarte, 2012), and covered with a crepe bandage and black bandana to minimise extraneous light (Canning & Scheutz, 2013; Hoshi et al., 2005). Each NIRS probe was connected by a single cable to a lightweight battery pack which was worn on an adjustable belt around the waist. Following the completion of NIRS device positioning the participants completed four resting Stroop tests and four exercise Stroop tests each separated by 5 minutes of rest (see Figure 16). Whilst the haemodynamic response to is known to continue for up to 12 seconds after the presentation of a cognitive stimulus (Boynton et al., 1996; Buckner et al., 1996; Canning & Scheutz, 2013), the exact period of time it takes for the response to return to pre-stimulus levels is unknown, however, five minutes was chosen for the rest period as cerebral blood flow changes in response to moderate intensity exercise have been shown to return to baseline levels after five minutes of rest (Byun et al., 2014).

Exercise Stroop tests commenced five minutes after the start of the exercise bout and all cycling was conducted at an exercise intensity of 90 % GET determined individually for each participant. When completing the exercise Stroop test participants assumed an upright seated position on the bike and the keyboard wireless keyboard was positioned in front of them by the researcher. Participants maintained a cadence of  $\geq 70$  rpm throughout the bout of cycling exercise. During the resting period HR was monitored to ensure in returned to resting levels ( $\pm 10$  bpm) during the period between trials.

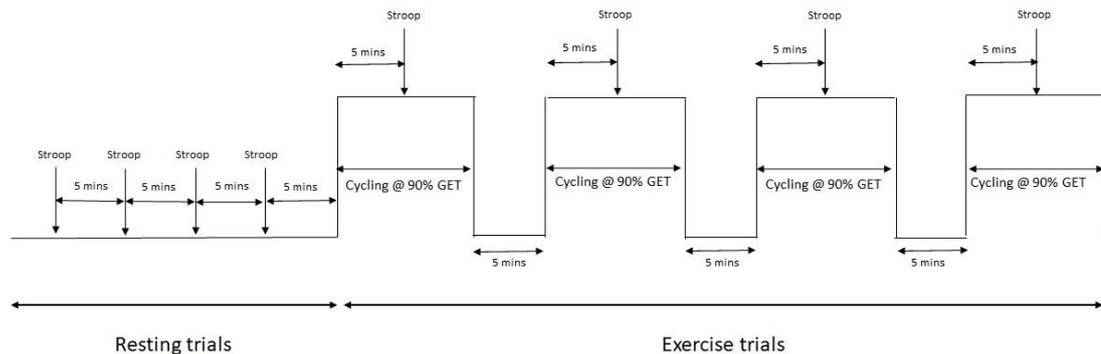


Figure 16: Protocol for NIRS sessions including Stroop tests at rest and during exercise on a cycle ergometer at 90% GET.

### 5.3.8 NIRS Data collection

Haemodynamic changes in response to the Stroop protocol were continuously monitored during the NIRS trials using the Artinis Portalite NIRS device (Artinis medical systems, Einsteinweg, The Netherlands). The full specifications for the device measurements can be found in section 4.3.9. The midpoint of the probe was situated over the relevant position (e.g., Fp1 or Fp2) to ensure the characteristic 'banana shaped' profile of light propagation (Ehlis et al., 2005; Gratton, 1994; Haessinger et al., 2011) included the region of interest. A sampling rate of 50 Hz was used during all trials.

### 5.3.9 EEG Experimental trial

During the EEG trial participants completed four resting Stroop trials whilst a 64 channel EEG signal was recorded continuously by active scalp measurements according to the extended 10-20 positioning system (Jasper, 1958). Electrodes were placed above and below both eyes and at the outer canthus of each eye in order to record vertical and horizontal

eye movements respectively. Data was recorded using BioSemi Active Two amplifiers (BioSemi B.V., Amsterdam, the Netherlands) and filtered between 0.6 and 100 Hz. A sampling rate of 512 Hz was used during all trials.

### **5.3.10 Data analysis**

#### *Behavioural Data*

The first resting and first exercise Stroop test from each NIRS trial and the first Stroop test from the EEG trial was considered a familiarisation trial and was not included for analysis. For the remainder of the Stroop tests percentage accuracy (ACC) and response time (RT) were recorded and averaged for resting, exercise and EEG congruent and incongruent trials. Where the participant failed to record a response during the 1200ms stimulus presentation a response time of 1.2s was recorded and the trial was marked as an incorrect response. A 3 (Trial) x 2 (Block) repeated measures ANOVA was used to compare Stroop response time (RT) between the AF3/AF4, Fp1/Fp2 and EEG trials at rest and to compare response times between the congruent and incongruent trials. A 3 (Trial) x 2 (Block) repeated measures ANOVA was also used to compare Stroop response accuracy (ACC). Greenhouse Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. Paired-t-tests were used to compare AF3/AF4 and Fp1/Fp2 congruent and incongruent RT and ACC at rest and during exercise. A Pearson's correlation test was also used to determine relationships between Stroop data and responses recorded by NIRS and EEG.

#### *NIRS Data*

Data was smoothed using a Gaussian filter (via the Artinis Oxysoft software), following which mean values for [O<sub>2</sub>Hb], [HHb], [tHb] and [TSI] were determined for congruent and incongruent trials and averaged for each NIRS position (e.g., AF3/AF4) for resting and exercise trials. A change in chromophore levels relative to a 10s baseline recorded prior to the start of each Stroop test was determined as described in chapter 4. Following this the relative values of [O<sub>2</sub>Hb], [HHb] were used to calculate [Hb<sub>diff</sub>] using the equation  $[Hb_{diff}] = ([O_2Hb] + [HHb])/2$ . All chromophore values were averaged for congruent and incongruent trials at rest and during exercise. A 4 (Position) x 2 (Rest) x 2 (Block) repeated measures ANOVA was used for [O<sub>2</sub>Hb], [HHb], [tHb], [TSI] and [Hb<sub>diff</sub>]. Greenhouse Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. Separate paired t-tests were used to interrogate

significant interactions between rest and position, between rest and block and between position and block. A Pearson's correlation was used to determine the relationship of each value to the behavioural data and EEG data as well as to determine any relationships between chromophore concentrations in the different positions.

#### *EEG Data*

EEG data were processed using the MATLAB based toolbox EEGLAB (Delorme & Makeig, 2004). The EEG data was initially algebraically re-referenced to the average of two earlobes and a filter between 0.5 and 30 Hz was applied to remove signal-noise interference. A more conservative filter than the 50Hz usually used (e.g., Jones & Bhattacharya, 2014) was chosen due to the electrodes of interest being positioned on the forehead and therefore more subject to movement artefacts from facial muscles. Each trial was divided into congruent and incongruent blocks and one second epochs were created from the data. All files were then visually inspected for movement artefacts and any epochs containing large artefacts due to muscle movements or eye blinks were rejected. Once visual inspection of the data was complete files were appended to create one congruent and one incongruent condition dataset. The Welch method (Welch, 1967) was then used to estimate the mean log spectrum of the data using the default EEGLAB settings of 2Hz, 0 overlap which gives the power spectra of 2Hz up to 30Hz where the filter was set. The power spectra were separately estimated for each electrode position (Fp1, Fp2, AF3 and AF4) and condition (congruent and incongruent) for each participant. Spectral power was then averaged for the following frequency bands: delta (1-3Hz), theta (4-7Hz), alpha (8-12Hz), beta-1 (13-21Hz) and beta-2 (22-30Hz).

A 4 (Position) x 5 (Frequency) x 2 (Block) repeated measures ANOVA was used to determine differences in response for each block at each of the frequencies for the four different positions. Greenhouse Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. Paired t-tests were used to examine significant interaction effects between Position x Block and Frequency x Block and separate one-way ANOVAs were used to investigate significant interactions between Position and Frequency. A Pearson's correlation was used to examine relationships between the EEG data and the behavioural and NIRS data.

The alpha level for all data analysis was set at  $p < 0.05$ . Data was presented as mean  $\pm$  SD and 95% confidence intervals (95% CI) were also reported where appropriate. Effect

sizes were interpreted as: small = 0.01, medium = 0.06, large = 0.14 according to guidelines from Cohen, Miles and Shevlin (2001).

## 5.6 Results

### 5.6.1 Behavioural data

#### *Stroop Response Time*

In the resting conditions there was a significant main effect of Trial,  $F(1.36,31.25) = 3.97$ ,  $p = 0.04$ ,  $\eta_p^2 = 0.15$  and of Block,  $F(1,23) = 32.11$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.58$ . Pairwise comparisons revealed that RT was significantly quicker in the EEG trials than the AF3/AF4 trials ( $p = 0.04$ ) and significantly quicker in the congruent than incongruent trials ( $p < 0.001$ ) (see Table 6). There were no significant interactions between Trial and Block,  $F(2,46) = 1.13$ ,  $p = 0.33$ ,  $\eta_p^2 = 0.05$ . Paired t-tests revealed that there were no significant differences in Stroop RT between Rest and Exercise trials in the FP1/Fp2 congruent,  $t = 1.08$ ,  $p = 0.29$  or incongruent blocks,  $t = 1.34$ ,  $p = 0.19$  or in the AF3/AF4 congruent,  $t = -1.34$ ,  $p = 0.19$  or incongruent blocks,  $t = -0.80$ ,  $p = 0.43$ .

Table 6: Stroop response time and percentage accuracy for the three different resting trials. Values presented as Mean ( $\pm$  SD)

Performance measure	EEG Congruent	EEG Incongruent	Fp1/Fp2 Congruent	Fp1/Fp2 Incongruent	AF3/AF4 Congruent	AF3/AF4 Incongruent
Response Time (s)	0.60 (0.11)	0.66 (0.08)	0.65 (0.12)	0.70 (0.09)	0.62 (0.11)	0.69 (0.09)
Response Accuracy (%)	96 (0.03)	94 (0.04)	98 (0.2)	96 (0.04)	95 (0.08)	93 (0.10)

*Note: The first two columns relate to EEG data and the final four columns to NIRS data*

#### *Stroop Response Accuracy*

In the resting conditions there was a significant main effect of Block,  $F(1,23) = 6.16$ ,  $p = 0.02$ ,  $\eta_p^2 = 0.21$ . Pairwise comparisons revealed that response accuracy was significantly

higher in the congruent than incongruent blocks ( $p = 0.02$ ) (see Table 6). There was no significant effect of Trial,  $F(1.13, 26.09) = 1.65$ ,  $p = 0.21$ ,  $\eta_p^2 = 0.07$  and there were no significant interactions between Trial and Block,  $F(2, 46) = 0.36$ ,  $p = 0.70$ ,  $\eta_p^2 = 0.02$ . Paired t-tests revealed that there were no significant differences in Stroop ACC between Rest and Exercise trials in the Fp1/Fp2 congruent,  $t = 1.68$ ,  $p = 0.11$  or incongruent blocks,  $t = 0.88$ ,  $p = 0.39$  or in the AF3/AF4 congruent,  $t = -0.86$ ,  $p = 0.40$  or incongruent blocks,  $t = -0.93$ ,  $p = 0.36$ .

### 5.6.2 EEG Data

The repeated measures ANOVA revealed a significant main effect for Position,  $F(3, 69) = 27.59$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.55$ . Pairwise comparisons revealed that power was higher in the AF3 position than the Fp1 position ( $p = 0.02$ ), the Fp2 position ( $p < 0.001$ ) and the AF4 position ( $p = 0.006$ ).

There was also a significant main effect for Frequency,  $F(1.77, 40.69) = 27.59$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.91$ . Power was also significantly higher in the Fp1 position than the Fp2 position ( $p < 0.001$ ) and in the AF4 position than the Fp2 position ( $p = 0.001$ ). Power was also higher in the Alpha frequency band than the Beta1 frequency band ( $p < 0.001$ ). Power was significantly higher in the Delta frequency band than the Alpha, Beta1, Beta2 and Theta frequency bands ( $p < 0.001$ ). Power was significantly higher in the Theta frequency band than the Alpha, Beta1 and Beta2 frequency bands ( $p < 0.001$ ). No significant main effect was observed for Block,  $F(1, 23) = 3.33$ ,  $p = 0.08$ ,  $\eta_p^2 = 0.13$ .

A significant Position x Frequency interaction was observed,  $F(4.31, 99.20) = 25.68$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.53$ . There were no significant interactions between Position and Block,  $F(3, 69) = 0.50$ ,  $p = 0.68$ ,  $\eta_p^2 = 0.02$ , or Frequency and Block,  $F(2.39, 54.85) = 2.29$ ,  $p = 0.10$ ,  $\eta_p^2 = 0.09$ .

#### *Position x Frequency Interaction*

Alpha power was significantly higher in the Fp1 position than the Fp2 position ( $p < 0.001$ ) and in the AF4 position than the Fp2 position ( $p < 0.001$ ). In the AF3 position Alpha power was significantly higher than in the Fp1, Fp2 and AF4 positions ( $p < 0.001$ ). Power in the Beta1 Frequency range was significantly higher in the Fp1 and AF4 positions than in the Fp2 position ( $p < 0.001$ ). Significantly higher power was also observed in the AF3 position than in the Fp1 ( $p = 0.001$ ), the Fp2 ( $p < 0.001$ ), and the AF4 positions ( $p = 0.004$ ). In the Beta2

frequency range power was significantly lower in the Fp2 position than in the Fp1 ( $p < 0.001$ ), AF4 ( $p = 0.01$ ), and AF3 positions ( $p = 0.001$ ). In the Delta frequency range power was significantly higher in the Fp1 position than in the AF3 ( $p < 0.001$ ), and AF4 positions ( $p < 0.001$ ). Power was also higher in the Fp2 position than in the AF4 position ( $p = 0.001$ ). In the Theta frequency range power was lower in the Fp2 position than in the Fp1, AF3, and AF4 positions ( $p < 0.001$ ). Theta power was higher in the AF3 position than in the Fp1 ( $p < 0.001$ ) and AF4 ( $p = 0.01$ ) positions.

*Correlations with Stroop Response Time (RT)*

Significant correlations were found between EEG data and Stroop RT during congruent and incongruent blocks in the Delta frequency range in the Fp1, Fp2 and AF4 positions, all other correlations between Stroop RT and EEG data were non-significant (see Table 7).

Table 7: Correlations between EEG data and Stroop response time

Position	Frequency Alpha		Beta1 Theta	Beta2	Delta
<i>Congruent</i>					
Fp1	$r = -0.09$ (0.68)	$r = 0.08$ (0.71)	$r = 0.02$ (0.93)	$r = 0.45$ (0.03)	$r = 0.09$ (0.67)
Fp2	$r = -0.03$ (0.90)	$r = 0.03$ (0.90)	$r = 0.05$ (0.81)	$r = 0.46$ (0.03)	$r = 0.05$ (0.84)
AF3	$r = -0.10$ (0.66)	$r = 0.03$ (0.88)	$r = 0.01$ (0.97)	$r = 0.40$ (0.06)	$r = 0.02$ (0.91)
AF4	$r = 0.07$ (0.75)	$r = 0.11$ (0.61)	$r = 0.09$ (0.68)	$r = 0.46$ (0.02)	$r = 0.26$ (0.23)
<i>Incongruent</i>					
Fp1	$r = -0.12$ (0.56)	$r = 0.06$ (0.77)	$r = 0.15$ (0.48)	$r = 0.43$ (0.04)	$r = -0.07$ (0.75)
Fp2	$r = -0.16$ (0.45)	$r = -0.13$ (0.53)	$r = 0.22$ (0.31)	$r = 0.38$ (0.07)	$r = 0.01$ (0.97)
AF3	$r = -0.22$ (0.31)	$r = -0.09$ (0.53)	$r = 0.05$ (0.82)	$r = 0.46$ (0.03)	$r = -0.03$ (0.88)
AF4	$r = -0.22$ (0.31)	$r = -0.02$ (0.94)	$r = 0.12$ (0.58)	$r = 0.44$ (0.03)	$r = 0.05$ (0.83)

*Note. Significance values (p values) presented in parentheses*

### Correlations with Stroop Accuracy (ACC)

In the congruent blocks significant negative correlations with Stroop ACC were found in the Fp1 and Fp2 positions at the Alpha, Beta1 and Theta frequency bands. In the AF4 position a significant negative correlation was found in the Alpha Frequency band. No other significant correlations were observed in the congruent blocks and no significant correlations were found in the incongruent blocks (see Table 8).

Table 8 Correlations between EEG data and Stroop accuracy

Position	Frequency				
	Alpha	Beta1	Beta2	Delta	Theta
<i>Congruent</i>					
Fp1	$r = -0.09$ (0.68)	$r = 0.08$ (0.71)	$r = 0.02$ (0.93)	$r = 0.45$ (0.03)	$r = 0.09$ (0.67)
Fp2	$r = -0.03$ (0.90)	$r = 0.03$ (0.90)	$r = 0.05$ (0.81)	$r = 0.46$ (0.03)	$r = 0.05$ (0.84)
AF3	$r = -0.10$ (0.66)	$r = 0.03$ (0.88)	$r = 0.01$ (0.97)	$r = 0.40$ (0.06)	$r = 0.02$ (0.91)
AF4	$r = 0.07$ (0.75)	$r = 0.11$ (0.61)	$r = 0.09$ (0.68)	$r = 0.46$ (0.02)	$r = 0.26$ (0.23)
<i>Incongruent</i>					
Fp1	$r = -0.12$ (0.56)	$r = 0.06$ (0.77)	$r = 0.15$ (0.48)	$r = 0.43$ (0.04)	$r = -0.07$ (0.75)
Fp2	$r = -0.16$ (0.45)	$r = -0.13$ (0.53)	$r = 0.22$ (0.31)	$r = 0.38$ (0.07)	$r = 0.01$ (0.97)
AF3	$r = -0.22$ (0.31)	$r = -0.09$ (0.53)	$r = 0.05$ (0.82)	$r = 0.46$ (0.03)	$r = -0.03$ (0.88)
AF4	$r = -0.22$ (0.31)	$r = -0.02$ (0.94)	$r = 0.12$ (0.58)	$r = 0.44$ (0.03)	$r = 0.05$ (0.83)

Note. Significance values ( $p$  values) presented in parentheses

### 5.6.3 NIRS Data

Examination of [tHb] values using a repeated measures ANOVA revealed that there were no significant main effects for Position,  $F(2.05,47.08) = 1.05$ ,  $p = 0.36$ ,  $\eta_p^2 = 0.04$  or Block,  $F(1,23) = 0.93$ ,  $p = 0.35$ ,  $\eta_p^2 = 0.04$ , however significant main effects were observed for Rest,  $F(1,23) = 10.53$   $p = 0.004$ ,  $\eta_p^2 = 0.31$ . Pairwise comparisons revealed that [tHb] values were significantly higher in the resting trials than the exercise trials ( $p = 0.004$ ). There were no significant interaction effects for Position x Rest,  $F(3,69) = 0.10$ ,  $p = 0.96$ ,  $\eta_p^2 = 0.004$ ,

Position x Block,  $F(2, 46.03) = 0.79$ ,  $p = 0.46$ ,  $\eta_p^2 = 0.03$  or Rest x Block,  $F(1,23) = 3.06$ ,  $p = 0.09$ ,  $\eta_p^2 = 0.12$ .

The repeated measures ANOVA for [TSI] revealed a significant main effect for Position,  $F(3,69) = 3.05$ ,  $p = 0.03$ ,  $\eta_p^2 = 0.12$  and Rest,  $F(1,23) = 66.50$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.74$ . Pairwise comparisons revealed the [TSI] values were significantly higher in the AF3 position than the Fp1 position ( $p = 0.03$ ) and significantly higher in the resting trials than the exercise trials ( $p < 0.001$ ). There were no significant main effects for Block,  $F(1,23) = 1.91$ ,  $p = 0.18$ ,  $\eta_p^2 = 0.08$ . No significant interactions were observed for Position x Rest,  $F(3,69) = 0.55$ ,  $p = 0.65$ ,  $\eta_p^2 = 0.02$  or Position x Block,  $F(2.35,54.08) = 1.72$ ,  $p = 0.17$ ,  $\eta_p^2 = 0.07$ . A significant interaction was observed for Rest x Block,  $F(1,23) = 47.38$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.67$ . Paired t-tests revealed that [TSI] values were significantly higher in the resting trials for congruent blocks,  $t = 6.74$ ,  $p < 0.001$  and incongruent blocks,  $t = 8.68$ ,  $p < 0.001$ .

For [O<sub>2</sub>Hb] values the repeated measures ANOVA revealed a significant main effect for Rest  $F(1,23) = 35.76$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.61$ . Pairwise comparisons showed significantly higher [O<sub>2</sub>Hb] values in the resting trials than the exercise trials ( $p < 0.001$ ). There were no significant main effects for position,  $F(2.31,53.15) = 0.85$ ,  $p = 0.45$ ,  $\eta_p^2 = 0.04$  or Block,  $F(1,23) = 2.05$ ,  $p = 0.17$ ,  $\eta_p^2 = 0.08$ . No significant interactions were observed for Position x Rest,  $F(3,69) = 0.40$ ,  $p = 0.75$ ,  $\eta_p^2 = 0.02$  or Position x Block,  $F(1.48,52.74) = 0.65$ ,  $p = 0.59$ ,  $\eta_p^2 = 0.03$ . A significant interaction was observed for Rest x Block,  $F(1,23) = 17.02$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.43$ . Paired t-tests revealed that [TSI] values were significantly higher in the resting trials for congruent blocks,  $t = 4.90$ ,  $p < 0.001$  and incongruent blocks,  $t = 6.44$ ,  $p < 0.001$ .

Examination of [HHb] values revealed that there was a significant main effect for Rest,  $F(1,23) = 10.36$ ,  $p = 0.004$ ,  $\eta_p^2 = 0.31$ . Pairwise comparisons revealed that [HHb] values were significantly higher during exercise than at rest ( $p = 0.004$ ). There were no significant main effects for Position,  $F(1.87,43.06) = 2.52$ ,  $p = 0.07$ ,  $\eta_p^2 = 0.10$ , or Block,  $F(1,23) = 1.73$ ,  $p = 0.20$ ,  $\eta_p^2 = 0.07$ . There were no significant interaction effects for Position x Rest,  $F(3,69) = 0.39$ ,  $p < 0.39$ ,  $\eta_p^2 = 0.04$ , Position x Block,  $F(2.12,48.78) = 2.83$ ,  $p < 0.07$ ,  $\eta_p^2 = 0.11$ . Significant interactions were observed for Rest x Block,  $F(1,23) = 18.97$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.45$ . Paired t-tests revealed that [HHb] values were significantly higher during exercise than at rest in the congruent,  $t = -2.48$ ,  $p = 0.02$  and incongruent blocks,  $t = -3.61$ ,  $p = 0.001$ .

For [Hb<sub>diff</sub>] values the repeated measures ANOVA revealed a significant main effect for Rest,  $F(1,23) = 68.89, p < 0.001, \eta_p^2 = 0.74$  and Block,  $F(1,23) = 6.65, p = 0.02, \eta_p^2 = 0.22$ . Pairwise comparisons showed significantly higher [Hb<sub>diff</sub>] values in the resting trials than the exercise trials ( $p < 0.001$ ) and significantly higher [Hb<sub>diff</sub>] values in the incongruent blocks than the congruent blocks ( $p = 0.02$ ). There were no significant main effects for Position,  $F(2.19,53.36) = 1.56, p = 0.21, \eta_p^2 = 0.06$ . No significant interactions were observed for Position x Rest,  $F(2.09,48.00) = 1.29, p = 0.29, \eta_p^2 = 0.05$  or Position x Block,  $F(1.89,43.48) = 1.41, p = 0.26, \eta_p^2 = 0.06$ . A significant interaction was observed for Rest x Block,  $F(1,23) = 42.89, p < 0.001, \eta_p^2 = 0.65$ . Paired t-tests revealed that [Hb<sub>diff</sub>] values were significantly higher in the resting trials for congruent blocks,  $t = 7.20, p < 0.001$  and incongruent blocks,  $t = 8.56, p < 0.001$ .

#### *Correlations with Stroop response time (RT)*

There were no significant correlations between the NIRS data and Stroop RT in the resting congruent and incongruent trials (see Table 9). In the exercise trials there were significant correlations between Stroop RT and [tHb], [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] values in the AF3 position and between Stroop RT and [tHb] values in the AF4 position (see Table 9). There were no significant correlations between Stroop RT and the NIRS data in the Fp1 or Fp2 positions (see Table 9).

#### *Correlations with Stroop accuracy (ACC)*

In the resting congruent trials there were significant correlations between Stroop ACC and [HHb] values in the AF3 position. There were also significant negative correlations between Stroop ACC and [TSI] and [Hb<sub>diff</sub>] in the AF3 position (see Table 10). There were no correlations between Stroop ACC and any other NIRS variables in the resting congruent trials (see Table 10). In the resting incongruent trials there was a significant correlation between Stroop ACC and [HHb] values and a significant negative correlation between Stroop ACC and [TSI] values in the AF3 position (see Table 10).

In the exercise trials there was a significant correlation in the Fp1 position between Stroop ACC and [tHb], [O<sub>2</sub>Hb] and [HHb] values and in the AF3 position between Stroop ACC and [HHb] values in both the congruent and incongruent blocks (see Table 10). There were no other significant correlations between Stroop ACC and NIRS data in the exercise trials.

Table 9 Correlations between NIRS data and Stroop response time at rest and during exercise

	Rest				Exercise			
	Fp1	Fp2	AF3	AF4	Fp1	Fp2	AF3	AF4
<i>Congruent</i>								
[tHb]	$r = -0.03$ (0.90)	$r = 0.24$ (0.25)	$r = 0.13$ (0.56)	$r = 0.13$ (0.53)	$r = 0.38$ (0.07)	$r = 0.10$ (0.63)	$r = 0.67$ (<0.001)	$r = 0.43$ (0.04)
[TSI]	$r = 0.01$ (0.97)	$r = 0.13$ (0.56)	$r = -0.12$ (0.59)	$r = 0.03$ (0.87)	$r = 0.13$ (0.55)	$r = 0.26$ (0.22)	$r = 0.26$ (0.22)	$r = 0.07$ (0.74)
[O <sub>2</sub> Hb]	$r = -0.04$ (0.86)	$r = 0.24$ (0.26)	$r = 0.11$ (0.60)	$r = 0.12$ (0.58)	$r = 0.38$ (0.06)	$r = 0.17$ (0.42)	$r = 0.64$ (0.001)	$r = 0.37$ (0.07)
[HHb]	$r = -0.001$ (1.00)	$r = 0.12$ (0.59)	$r = 0.09$ (0.67)	$r = 0.10$ (0.65)	$r = 0.33$ (0.12)	$r = -0.13$ (0.56)	$r = 0.26$ (0.22)	$r = 0.15$ (0.49)
[Hb <sub>diff</sub> ]	$r = -0.04$ (0.86)	$r = 0.21$ (0.33)	$r = 0.07$ (0.76)	$r = 0.09$ (0.69)	$r = 0.28$ (0.18)	$r = 0.22$ (0.31)	$r = 0.53$ (0.01)	$r = 0.29$ (0.17)
<i>Incongruent</i>								
[tHb]	$r = -0.29$ (0.17)	$r = 0.16$ (0.45)	$r = 0.02$ (0.92)	$r = 0.09$ (0.68)	$r = 0.31$ (0.15)	$r = -0.03$ (0.87)	$r = 0.59$ (0.003)	$r = 0.07$ (0.76)
[TSI]	$r = -0.14$ (0.51)	$r = 0.07$ (0.75)	$r = 0.05$ (0.81)	$r = 0.03$ (0.90)	$r = 0.13$ (0.56)	$r = 0.18$ (0.40)	$r = 0.27$ (0.20)	$r = -0.13$ (0.53)
[O <sub>2</sub> Hb]	$r = -0.34$ (0.10)	$r = 0.16$ (0.47)	$r = 0.05$ (0.85)	$r = 0.09$ (0.69)	$r = 0.26$ (0.22)	$r = 0.05$ (0.81)	$r = 0.58$ (0.003)	$r = 0.05$ (0.83)
[HHb]	$r = -0.13$ (0.54)	$r = 0.06$ (0.79)	$r = -0.03$ (0.88)	$r = 0.04$ (0.84)	$r = 0.34$ (0.11)	$r = -0.20$ (0.35)	$r = 0.23$ (0.29)	$r = 0.11$ (0.62)
[Hb <sub>diff</sub> ]	$r = -0.38$ (0.07)	$r = 0.13$ (0.55)	$r = 0.06$ (0.78)	$r = -0.02$ (0.94)	$r = 0.12$ (0.57)	$r = 0.16$ (0.46)	$r = 0.49$ (0.02)	$r = 0.02$ (0.94)

Note. Significance values ( $p$  values) presented in parentheses

Table 10 Correlations between NIRS data and Stroop accuracy at rest and during exercise

	Rest				Exercise			
	Fp1	Fp2	AF3	AF4	Fp1	Fp2	AF3	AF4
<i>Congruent</i>								
[tHb]	$r = 0.02 (0.94)$	$r = -0.07 (0.75)$	$r = -0.02 (0.94)$	$r = 0.03 (0.88)$	$r = 0.53 (0.01)$	$r = -0.27 (0.20)$	$r = 0.21 (0.33)$	$r = 0.12 (0.57)$
[TSI]	$r = -0.09 (0.69)$	$r = 0.01 (0.95)$	$r = -0.59 (0.002)$	$r = -0.35 (0.09)$	$r = 0.07 (0.73)$	$r = -0.17 (0.43)$	$r = -0.31 (0.14)$	$r = -0.07 (0.75)$
[O <sub>2</sub> Hb]	$r = -0.03 (0.89)$	$r = -0.04 (0.85)$	$r = -0.24 (0.26)$	$r = -0.05 (0.82)$	$r = 0.50 (0.01)$	$r = -0.27 (0.21)$	$r = 0.04 (0.85)$	$r = 0.08 (0.71)$
[HHb]	$r = 0.09 (0.70)$	$r = -0.12 (0.59)$	$r = 0.42 (0.04)$	$r = 0.25 (0.24)$	$r = 0.50 (0.01)$	$r = -0.09 (0.69)$	$r = 0.50 (0.01)$	$r = 0.12 (0.58)$
[Hb <sub>diff</sub> ]	$r = -0.09 (0.67)$	$r = -0.002 (0.99)$	$r = -0.47 (0.02)$	$r = -0.14 (0.52)$	$r = 0.30 (0.16)$	$r = -0.22 (0.30)$	$r = -0.14 (0.50)$	$r = 0.04 (0.87)$
<i>Incongruent</i>								
[tHb]	$r = 0.03 (0.88)$	$r = 0.16 (0.45)$	$r = 0.13 (0.54)$	$r = 0.09 (0.68)$	$r = 0.52 (0.01)$	$r = -0.26 (0.23)$	$r = 0.15 (0.49)$	$r = 0.10 (0.63)$
[TSI]	$r = -0.08 (0.70)$	$r = 0.26 (0.22)$	$r = -0.50 (0.01)$	$r = -0.15 (0.49)$	$r = 0.32 (0.13)$	$r = -0.08 (0.72)$	$r = -0.38 (0.07)$	$r = -0.21 (0.32)$
[O <sub>2</sub> Hb]	$r = 0.01 (0.98)$	$r = 0.21 (0.33)$	$r = -0.03 (0.89)$	$r = 0.05 (0.80)$	$r = 0.50 (0.01)$	$r = -0.23 (0.29)$	$r = -0.04 (0.86)$	$r = 0.04 (0.85)$
[HHb]	$r = 0.08 (0.71)$	$r = -0.07 (0.73)$	$r = 0.45 (0.03)$	$r = 0.12 (0.57)$	$r = 0.48 (0.02)$	$r = -0.17 (0.42)$	$r = 0.52 (0.01)$	$r = 0.27 (0.21)$
[Hb <sub>diff</sub> ]	$r = -0.05 (0.82)$	$r = 0.22 (0.30)$	$r = -0.24 (0.26)$	$r = 0.06 (0.78)$	$r = 0.35 (0.09)$	$r = -0.18 (0.41)$	$r = -0.25 (0.25)$	$r = -0.05 (0.82)$

Note. Significance values ( $p$  values) presented in parentheses

#### 5.6.4 Correlations between EEG and NIRS data

*Congruent blocks.* In the congruent blocks there were significant correlations between the EEG and NIRS data in the AF3 position between [Hb<sub>diff</sub>] in the Delta frequency band and between [HHb] and power in the Delta frequency band. There was also a trend towards significance in the Theta frequency band (see Table 12). There were significant correlations in the AF4 position between [tHb] in the Theta frequency band (see Table 14). No significant correlations were found between the EEG and NIRS data in the Fp1 (see Table 11) and Fp2 (see Table 12) positions.

Table 11 Correlations between EEG and NIRS data in the Fp1 position

Chromophore	Frequency				
	Alpha	Beta1	Beta2	Delta	Theta
<i>Congruent</i>					
[tHb]	$r = -0.33$ (0.12)	$r = -0.05$ (0.80)	$r = 0.06$ (0.77)	$r = 0.07$ (0.76)	$r = -0.28$ (0.19)
[TSI]	$r = -0.08$ (0.72)	$r = -0.21$ (0.33)	$r = -0.11$ (0.62)	$r = -0.01$ (0.95)	$r = -0.08$ (0.71)
[O <sub>2</sub> Hb]	$r = -0.32$ (0.13)	$r = -0.11$ (0.60)	$r = 0.03$ (0.89)	$r = 0.06$ (0.78)	$r = -0.30$ (0.16)
[HHb]	$r = -0.22$ (0.30)	$r = 0.05$ (0.81)	$r = 0.09$ (0.68)	$r = 0.05$ (0.81)	$r = -0.15$ (0.49)
[Hb <sub>diff</sub> ]	$r = -0.17$ (0.42)	$r = -0.16$ (0.47)	$r = -0.04$ (0.87)	$r = 0.03$ (0.91)	$r = -0.21$ (0.34)
<i>Incongruent</i>					
[tHb]	$r = -0.06$ (0.78)	$r = 0.004$ (0.99)	$r = 0.02$ (0.91)	$r = 0.26$ (0.22)	$r = -0.29$ (0.17)
[TSI]	$r = -0.10$ (0.64)	$r = -0.19$ (0.39)	$r = -0.04$ (0.85)	$r = 0.19$ (0.39)	$r = -0.17$ (0.44)
[O <sub>2</sub> Hb]	$r = -0.05$ (0.82)	$r = -0.01$ (0.97)	$r = 0.03$ (0.88)	$r = 0.28$ (0.19)	$r = -0.31$ (0.14)
[HHb]	$r = -0.07$ (0.75)	$r = 0.03$ (0.90)	$r = 0.01$ (0.98)	$r = 0.18$ (0.41)	$r = -0.20$ (0.34)
[Hb <sub>diff</sub> ]	$r = -0.02$ (0.93)	$r = -0.03$ (0.89)	$r = 0.04$ (0.85)	$r = 0.26$ (0.22)	$r = -0.29$ (0.17)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 12 Correlations between EEG and NIRS data in the Fp2 position

Chromophore	Frequency				
	Alpha	Beta1	Beta2	Delta	Theta
<i>Congruent</i>					
[tHb]	$r = -0.01$ (0.95)	$r = 0.03$ (0.89)	$r = -0.03$ (0.88)	$r = -0.11$ (0.62)	$r = 0.19$ (0.38)
[TSI]	$r = -0.14$ (0.53)	$r = -0.16$ (0.45)	$r = -0.18$ (0.40)	$r = 0.06$ (0.79)	$r = 0.23$ (0.29)
[O <sub>2</sub> Hb]	$r = -0.05$ (0.83)	$r = -0.03$ (0.89)	$r = -0.09$ (0.68)	$r = -0.05$ (0.81)	$r = 0.23$ (0.27)
[HHb]	$r = 0.09$ (0.66)	$r = 0.20$ (0.36)	$r = 0.15$ (0.47)	$r = -0.21$ (0.33)	$r = -0.05$ (0.81)
[Hb <sub>diff</sub> ]	$r = -0.08$ (0.70)	$r = -0.10$ (0.64)	$r = -0.15$ (0.50)	$r = 0.02$ (0.93)	$r = 0.26$ (0.22)
<i>Incongruent</i>					
[tHb]	$r = -0.10$ (0.63)	$r = -0.05$ (0.83)	$r = -0.05$ (0.82)	$r = -0.21$ (0.32)	$r = 0.15$ (0.49)
[TSI]	$r = -0.12$ (0.58)	$r = -0.16$ (0.44)	$r = -0.17$ (0.42)	$r = 0.04$ (0.85)	$r = 0.20$ (0.34)
[O <sub>2</sub> Hb]	$r = -0.12$ (0.58)	$r = -0.11$ (0.60)	$r = -0.13$ (0.55)	$r = -0.14$ (0.53)	$r = 0.21$ (0.33)
[HHb]	$r = 0.02$ (0.94)	$r = 0.16$ (0.45)	$r = 0.20$ (0.34)	$r = -0.26$ (0.23)	$r = -0.13$ (0.56)
[Hb <sub>diff</sub> ]	$r = -0.13$ (0.54)	$r = -0.20$ (0.35)	$r = -0.23$ (0.29)	$r = -0.03$ (0.88)	$r = 0.24$ (0.26)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

*Incongruent blocks*

In the AF3 position power in the Delta frequency band was significantly correlated with [tHb], [O<sub>2</sub>Hb] and [HHb], and power in the Theta frequency band was significantly correlated with [HHb] (see Table 13). In the AF4 position power in the Delta frequency band was significantly correlated with [Hb<sub>diff</sub>], and power in the Theta frequency band was significantly correlated with [tHb], [O<sub>2</sub>Hb], and there was a trend towards a significant correlation with [Hb<sub>diff</sub>] (see Table 14). No significant correlations between the EEG and NIRS data were observed in the Fp1 (see Table 11) or Fp2 (see Table 12) positions.

Table 13 Correlations between EEG and NIRS data in the AF3 position

Chromophore	Frequency				
	Alpha	Beta1	Beta2	Delta	Theta
<i>Congruent</i>					
[tHb]	$r = 0.07$ (0.76)	$r = 0.02$ (0.93)	$r = 0.17$ (0.42)	$r = 0.39$ (0.06)	$r = 0.34$ (0.11)
[TSI]	$r = -0.32$ (0.13)	$r = -0.23$ (0.29)	$r = -0.12$ (0.57)	$r = -0.20$ (0.35)	$r = -0.21$ (0.34)
[O <sub>2</sub> Hb]	$r = -0.06$ (0.78)	$r = -0.07$ (0.74)	$r = 0.09$ (0.69)	$r = 0.27$ (0.21)	$r = 0.21$ (0.32)
[HHb]	$r = 0.28$ (0.19)	$r = 0.19$ (0.38)	$r = 0.26$ (0.22)	$r = 0.42$ (0.04)	<u><math>r = 0.41</math> (0.05)</u>
[Hb <sub>diff</sub> ]	$r = 0.18$ (0.40)	$r = 0.10$ (0.65)	$r = 0.15$ (0.48)	$r = 0.50$ (0.01)	<u><math>r = 0.23</math> (0.29)</u>
<i>Incongruent</i>					
[tHb]	$r = -0.25$ (0.25)	$r = -0.28$ (0.18)	$r = -0.11$ (0.61)	$r = -0.07$ (0.74)	$r = -0.38$ (0.07)
[TSI]	$r = 0.11$ (0.61)	$r = 0.02$ (0.94)	$r = 0.12$ (0.59)	$r = 0.41$ (0.04)	$r = 0.08$ (0.70)
[O <sub>2</sub> Hb]	$r = 0.25$ (0.24)	$r = 0.24$ (0.26)	$r = 0.16$ (0.46)	$r = 0.45$ (0.03)	$r = 0.45$ (0.03)
[HHb]	$r = 0.003$ (0.99)	$r = 0.16$ (0.45)	$r = 0.20$ (0.34)	$r = -0.26$ (0.23)	$r = -0.13$ (0.56)
[Hb <sub>diff</sub> ]	$r = -0.21$ (0.33)	$r = -0.09$ (0.67)	$r = 0.05$ (0.81)	$r = 0.24$ (0.27)	<u><math>r = -0.12</math> (0.58)</u>

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 14 Correlations between EEG and NIRS data in the AF4 position

Chromophore	Frequency				
	Alpha	Beta1	Beta2	Delta	Theta
<i>Congruent</i>					
[tHb]	$r = 0.19$ (0.37)	$r = 0.09$ (0.69)	$r = 0.09$ (0.69)	$r = 0.29$ (0.17)	$r = 0.42$ (0.04)
[TSI]	$r = 0.14$ (0.51)	$r = -0.01$ (0.96)	$r = -0.13$ (0.54)	$r = 0.25$ (0.24)	$r = 0.23$ (0.29)
[O <sub>2</sub> Hb]	$r = 0.21$ (0.32)	$r = 0.07$ (0.75)	$r = 0.02$ (0.92)	$r = 0.33$ (0.12)	$r = 0.39$ (0.06)
[HHb]	$r = 0.03$ (0.90)	$r = 0.09$ (0.67)	$r = 0.22$ (0.29)	$r = 0.003$ (0.99)	<u><math>r = 0.28</math> (0.19)</u>
[Hb <sub>diff</sub> ]	$r = -0.21$ (0.33)	$r = 0.04$ (0.86)	$r = -0.06$ (0.79)	$r = 0.34$ (0.14)	<u><math>r = 0.30</math> (0.16)</u>
<i>Incongruent</i>					
[tHb]	$r = 0.11$ (0.61)	$r = 0.04$ (0.87)	$r = 0.08$ (0.70)	$r = 0.29$ (0.16)	$r = 0.46$ (0.03)
[TSI]	$r = 0.19$ (0.38)	$r = 0.05$ (0.80)	$r = -0.14$ (0.52)	$r = 0.36$ (0.09)	$r = 0.29$ (0.17)
[O <sub>2</sub> Hb]	$r = 0.17$ (0.42)	$r = 0.06$ (0.79)	$r = 0.02$ (0.92)	$r = 0.39$ (0.06)	$r = 0.45$ (0.03)
[HHb]	$r = -0.11$ (0.61)	$r = -0.04$ (0.85)	$r = 0.19$ (0.38)	$r = -0.11$ (0.61)	$r = 0.21$ (0.33)
[Hb <sub>diff</sub> ]	$r = 0.27$ (0.20)	$r = 0.08$ (0.71)	$r = -0.07$ (0.75)	$r = 0.46$ (0.02)	$r = 0.39$ (0.06)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 15 Chromophore concentrations during resting trials for each position. Mean ( $\pm$ SD) values presented in Arbitrary Units (A.U.)

<i>Position</i>	<i>Congruent</i>					<i>Incongruent</i>				
	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hbdiff]	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hbdiff]
AF3	0.36(1.40)	0.22(0.76)	0.40(1.11)	-0.04(0.57)	0.22(0.54)	0.55(1.87)	0.46(0.76)	0.75(1.53)	-0.21(0.66)	0.48(0.72)
AF4	0.94(1.85)	0.43(0.67)	1.02(1.63)	-0.08(0.56)	0.55(0.79)	1.19(2.37)	0.59(0.73)	1.38(2.02)	-0.19(0.81)	1.09(1.24)
Fp1	-0.21(2.21)	0.48(0.95)	0.15(1.54)	-0.36(1.06)	0.26(0.73)	0.15(1.85)	0.83(0.91)	0.70(2.59)	-0.55(1.32)	0.63(0.93)
Fp2	0.60(2.92)	0.33(0.80)	0.77(2.55)	-0.17(0.85)	0.47(1.22)	0.79(3.40)	0.64(1.15)	1.22(3.12)	-0.44(1.18)	1.11(2.16)

Table 16 Chromophore concentrations during exercise trials for each position. Mean ( $\pm$ SD) values presented in Arbitrary Units (A.U.)

<i>Position</i>	<i>Congruent</i>					<i>Incongruent</i>				
	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hbdiff]	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hbdiff]
AF3	-0.38(1.40)	-0.39(0.59)	-0.58(1.27)	0.20(0.48)	-0.39(0.65)	-0.71(2.10)	-0.64(0.83)	-0.98(1.83)	0.27(0.74)	-0.63(0.92)
AF4	-0.07(1.24)	-0.24(0.53)	-0.30(1.27)	0.23(0.42)	-0.27(0.71)	0.40(2.63)	-0.40(0.66)	-0.16(2.21)	0.56(0.68)	-0.36(0.97)
Fp1	-0.45(2.46)	0.05(0.61)	-0.24(1.62)	-0.22(0.90)	-0.14(0.53)	-0.80(3.58)	-0.01(0.86)	-0.43(2.53)	-0.37(1.29)	-0.30(0.90)
Fp2	-0.31(1.54)	-0.11(0.48)	-0.29(1.36)	-0.02(0.59)	-0.14(0.71)	-0.06(2.36)	-0.17(0.68)	-0.14(1.97)	0.08(0.91)	-0.14(1.33)

### 5.6.5 Hemispheric correlations in NIRS data

#### *Resting trials*

In the congruent trials there were significant negative correlations between [TSI] values in the AF3 position and [HHb] values in the AF4 position and between [HHb] values in the AF3 and AF4 positions. No other significant correlations were observed between the AF3 and AF4 positions (see Table 18). There were no significant correlations between the Fp1 and Fp2 positions in the congruent resting trials (see Table 17). In the incongruent trials there were significant correlations between [tHb] values in the AF3 and AF4 positions, between [O<sub>2</sub>Hb] values in the AF3 and AF4 positions, and between [Hb<sub>diff</sub>] values in the AF3 and AF4 positions. Significant correlations were also observed between [tHb] values in the AF3 position and [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] values in the AF4 position, between [O<sub>2</sub>Hb] values in the AF3 position and [Hb<sub>diff</sub>] values in the AF4 position, and between [HHb] values in the AF3 position and [tHb] values in the AF4 position (see Table 20).

Negative correlations were found between [TSI] values in the Fp1 position and [HHb] values in the Fp2 position. No other significant correlations were observed between the Fp1 and Fp2 positions (see Table 18).

Table 17 Correlations between the Fp1 and Fp2 positions in the congruent resting trials

		Fp1				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
Fp2	[tHb]	$r = 0.28$ (0.18)	$r = 0.002$ (0.99)	$r = 0.23$ (0.27)	$r = 0.25$ (0.24)	$r = 0.07$ (0.76)
	[TSI]	$r = 0.18$ (0.41)	$r = 0.29$ (0.17)	$r = 0.24$ (0.27)	$r = 0.02$ (0.92)	$r = 0.23$ (0.28)
	[O <sub>2</sub> Hb]	$r = 0.26$ (0.23)	$r = 0.09$ (0.67)	$r = 0.23$ (0.27)	$r = 0.20$ (0.36)	$r = 0.10$ (0.63)
	[HHb]	$r = 0.20$ (0.35)	$r = -0.26$ (0.21)	$r = 0.10$ (0.63)	$r = 0.27$ (0.20)	$r = -0.09$ (0.68)
	[Hb <sub>diff</sub> ]	$r = 0.20$ (0.35)	$r = 0.19$ (0.38)	$r = 0.21$ (0.33)	$r = 0.11$ (0.61)	$r = 0.14$ (0.52)

*Note* Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 18 Correlations between the Fp1 and Fp2 positions in the incongruent resting trials

		Fp1				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
Fp2	[tHb]	$r = -0.06$ (0.79)	$r = -0.16$ (0.45)	$r = -0.10$ (0.64)	$r = 0.04$ (0.87)	$r = -0.16$ (0.44)
	[TSI]	$r = 0.11$ (0.62)	$r = 0.30$ (0.15)	$r = 0.13$ (0.56)	$r = 0.06$ (0.80)	$r = 0.13$ (0.53)
	[O <sub>2</sub> Hb]	$r = -0.03$ (0.89)	$r = -0.01$ (0.97)	$r = -0.06$ (0.79)	$r = 0.03$ (0.90)	$r = -0.10$ (0.65)
	[HHb]	$r = -0.09$ (0.69)	$r = -0.45$ (0.03)	$r = -0.14$ (0.52)	$r = 0.03$ (0.88)	$r = -0.21$ (0.32)
	[Hb <sub>diff</sub> ]	$r = 0.01$ (0.98)	$r = 0.15$ (0.47)	$r = -0.01$ (0.98)	$r = 0.02$ (0.91)	$r = -0.03$ (0.91)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 19 Correlations between the AF3 and AF4 positions in the congruent resting trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
AF4	[tHb]	$r = 0.19$ (0.39)	$r = -0.24$ (0.25)	$r = 0.06$ (0.79)	$r = 0.34$ (0.10)	$r = -0.12$ (0.58)
	[TSI]	$r = -0.11$ (0.62)	$r = 0.25$ (0.24)	$r = 0.04$ (0.86)	$r = -0.34$ (0.11)	$r = 0.22$ (0.31)
	[O <sub>2</sub> Hb]	$r = 0.14$ (0.52)	$r = -0.13$ (0.55)	$r = 0.08$ (0.71)	$r = 0.18$ (0.39)	$r = -0.02$ (0.95)
	[HHb]	$r = 0.21$ (0.33)	$r = -0.44$ (0.03)	$r = -0.04$ (0.84)	$r = 0.59$ (0.002)	$r = -0.36$ (0.09)
	[Hb <sub>diff</sub> ]	$r = 0.07$ (0.74)	$r = 0.02$ (0.92)	$r = 0.10$ (0.65)	$r = -0.02$ (0.93)	$r = 0.11$ (0.61)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 20 Correlations between the AF3 and AF4 positions in the incongruent resting trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
AF4	[tHb]	$r = 0.44$ (0.03)	$r = -0.03$ (0.89)	$r = 0.36$ (0.08)	$r = 0.42$ (0.04)	$r = 0.20$ (0.36)
	[TSI]	$r = 0.24$ (0.25)	$r = 0.28$ (0.19)	$r = 0.30$ (0.15)	$r = -0.01$ (0.96)	$r = 0.33$ (0.12)
	[O <sub>2</sub> Hb]	$r = 0.50$ (0.01)	$r = 0.06$ (0.79)	$r = 0.46$ (0.02)	$r = 0.36$ (0.08)	$r = 0.33$ (0.12)
	[HHb]	$r = 0.04$ (0.85)	$r = -0.23$ (0.28)	$r = -0.09$ (0.68)	$r = 0.32$ (0.13)	$r = -0.24$ (0.26)
	[Hb <sub>diff</sub> ]	$r = 0.49$ (0.02)	$r = 0.16$ (0.16)	$r = 0.49$ (0.02)	$r = 0.25$ (0.24)	$r = 0.41$ (0.05)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

### Exercise trials

In the congruent blocks there were significant correlations between the [TSI] values in the Fp1 and Fp2 positions. There were also significant negative correlations between [HHb] values in the Fp1 position and [O<sub>2</sub>Hb] values in the Fp2 position and between [HHb] values in the Fp1 position and [Hb<sub>diff</sub>] values in the Fp2 position (see Table 21). There were no other significant correlations between the Fp1 and Fp2 positions in the congruent resting trials.

AF3 and AF4 positions for [O<sub>2</sub>Hb] values (see Figure 17), [tHb] values (see Figure 18), [TSI] values (see Figure 19), [HHb] values (see Figure 20) and [Hb<sub>diff</sub>] values (see Figure 21).

Significant correlations were also observed between [tHb] values in the AF3 position and [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] values in the AF4 position, between [TSI] values in the AF3 position and [tHb], [O<sub>2</sub>Hb], and [Hb<sub>diff</sub>] values in the AF4 position, between [O<sub>2</sub>Hb] values in the AF3 position and [tHb] values, [TSI] values and [Hb<sub>diff</sub>] values in the AF4 position and between [Hb<sub>diff</sub>] values in the AF3 position and [tHb], [TSI] and [O<sub>2</sub>Hb] values in the AF4 position. There were also significant negative correlations between [HHb] values in the AF3 position and [TSI] values in the AF4 position. All correlation values are presented in Table 22.

Table 21 Correlations between the Fp1 and Fp2 positions in the congruent exercise trials

		Fp1				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
Fp2	[tHb]	$r = -0.34$ (0.11)	$r = 0.10$ (0.64)	$r = -0.31$ (0.14)	$r = -0.34$ (0.11)	$r = -0.15$ (0.48)
	[TSI]	$r = -0.18$ (0.39)	$r = 0.43$ (0.04)	$r = -0.07$ (0.75)	$r = -0.34$ (0.10)	$r = 0.21$ (0.32)
	[O <sub>2</sub> Hb]	$r = -0.37$ (0.08)	$r = 0.25$ (0.24)	$r = -0.30$ (0.16)	$r = -0.44$ (0.03)	$r = -0.05$ (0.83)
	[HHb]	$r = -0.03$ (0.87)	$r = -0.31$ (0.14)	$r = -0.12$ (0.57)	$r = 0.12$ (0.59)	$r = -0.29$ (0.16)
	[Hb <sub>diff</sub> ]	$r = -0.34$ (0.11)	$r = 0.37$ (0.08)	$r = -0.23$ (0.28)	$r = -0.47$ (0.02)	$r = 0.08$ (0.71)

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 22 Correlations between the AF3 and AF4 positions in the congruent exercise trials

		Fp1				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
Fp2	[tHb]	$r = 0.68 (< 0.001)$	$r = 0.52 (0.01)$	$r = 0.75 (< 0.001)$	$r = 0.01 (0.98)$	$r = 0.73 (< 0.001)$
	[TSI]	$r = 0.21 (0.33)$	$r = 0.73 (< 0.001)$	$r = 0.45 (0.03)$	$r = -0.57 (0.004)$	$r = 0.64 (0.001)$
	[O <sub>2</sub> Hb]	$r = 0.60 (0.002)$	$r = 0.64 (0.001)$	$r = 0.74 (< 0.001)$	$r = -0.21 (0.33)$	$r = 0.80 (< 0.001)$
	[HHb]	$r = 0.20 (0.36)$	$r = -0.39 (0.06)$	$r = -0.03 (0.90)$	$r = 0.64 (0.001)$	$r = -0.26 (0.22)$
	[Hb <sub>diff</sub> ]	$r = 0.48 (0.02)$	$r = 0.69 (< 0.001)$	$r = 0.67 (< 0.001)$	$r = -0.37 (0.07)$	$r = 0.79 (< 0.001)$

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 23 Correlations between the Fp1 and Fp2 positions in the incongruent exercise trials

		Fp1				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
Fp2	[tHb]	$r = -0.42 (0.04)$	$r = 0.09 (0.68)$	$r = -0.36 (0.08)$	$r = -0.45 (0.03)$	$r = -0.19 (0.39)$
	[TSI]	$r = -0.14 (0.53)$	$r = 0.25 (0.24)$	$r = -0.06 (0.77)$	$r = -0.25 (0.24)$	$r = 0.09 (0.67)$
	[O <sub>2</sub> Hb]	$r = -0.43 (0.04)$	$r = 0.16 (0.45)$	$r = -0.35 (0.10)$	$r = -0.51 (0.01)$	$r = -0.13 (0.55)$
	[HHb]	$r = -0.16 (0.46)$	$r = -0.13 (0.56)$	$r = -0.18 (0.39)$	$r = -0.08 (0.72)$	$r = -0.20 (0.35)$
	[Hb <sub>diff</sub> ]	$r = -0.39 (0.06)$	$r = 0.19 (0.38)$	$r = -0.31 (0.14)$	$r = -0.48 (0.02)$	$r = -0.09 (0.68)$

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

Table 24 Correlations between the AF3 and AF4 positions in the incongruent exercise trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
AF4	[tHb]	$r = 0.35 (0.09)$	$r = 0.33 (0.12)$	$r = 0.39 (0.06)$	$r = 0.02 (0.92)$	$r = 0.38 (0.06)$
	[TSI]	$r = 0.03 (0.91)$	$r = 0.64 (0.001)$	$r = 0.24 (0.27)$	$r = -0.52 (0.01)$	$r = 0.44 (0.03)$
	[O <sub>2</sub> Hb]	$r = 0.33 (0.12)$	$r = 0.43 (0.04)$	$r = 0.42 (0.04)$	$r = -0.12 (0.58)$	$r = 0.47 (0.002)$
	[HHb]	$r = 0.30 (0.16)$	$r = -0.14 (0.51)$	$r = 0.15 (0.48)$	$r = 0.47 (0.02)$	$r = -0.04 (0.87)$
	[Hb <sub>diff</sub> ]	$r = 0.27 (0.20)$	$r = 0.55 (0.01)$	$r = 0.43 (0.04)$	$r = -0.30 (0.16)$	$r = 0.55 (0.01)$

Note Significance values ( $p$  values) presented in parentheses after  $r$  values

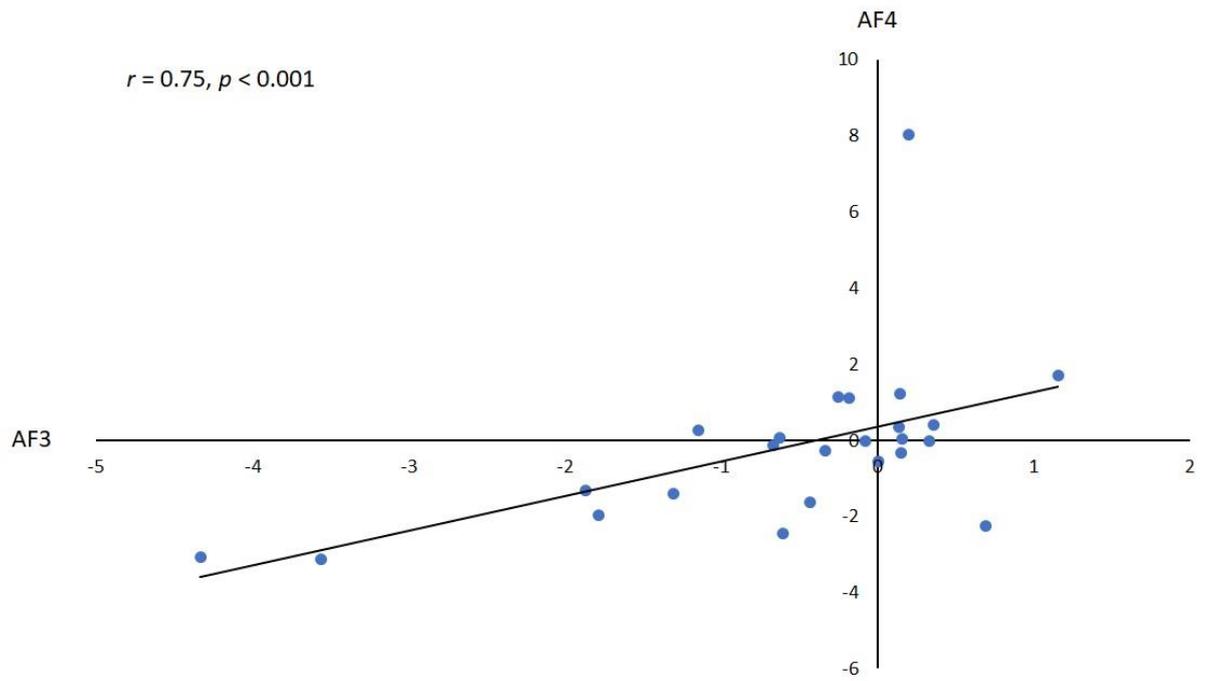


Figure 17; Relationship between the AF3 and AF4 positions for [O2Hb] in congruent blocks during exercise. *Note* Data presented in arbitrary units (A.U.)

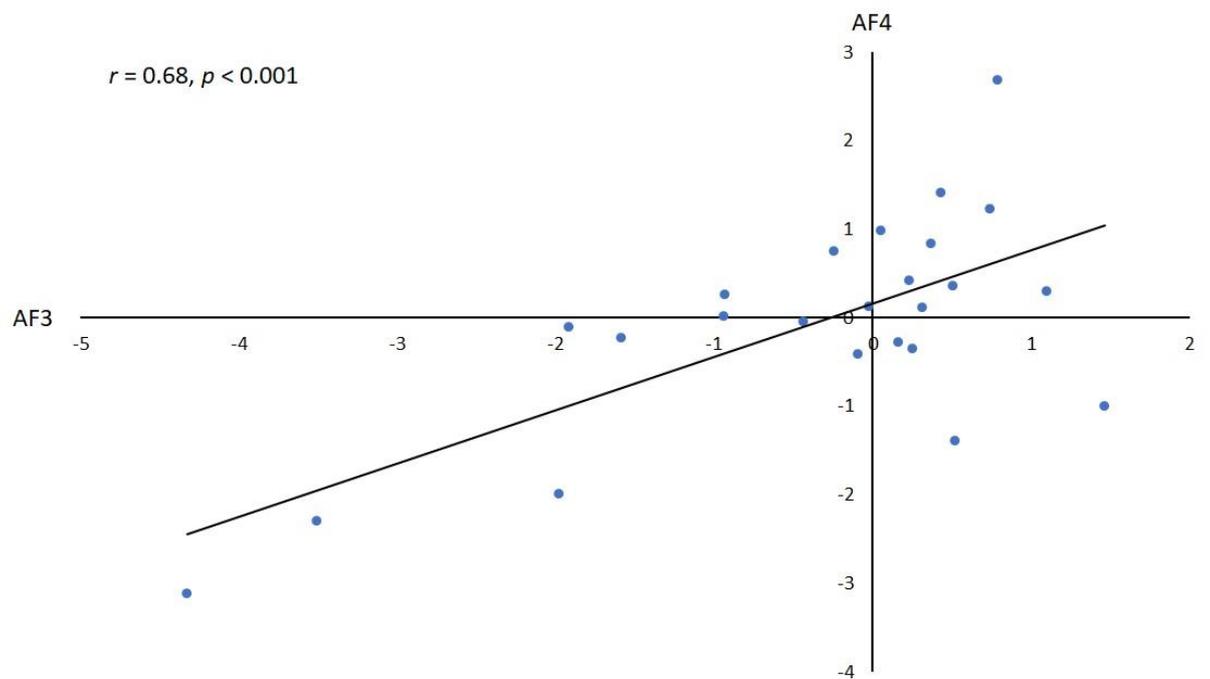


Figure 18: Relationship between the AF3 and AF4 positions for [tHb] in congruent blocks during exercise. *Note* Data presented in arbitrary units (A.U.)

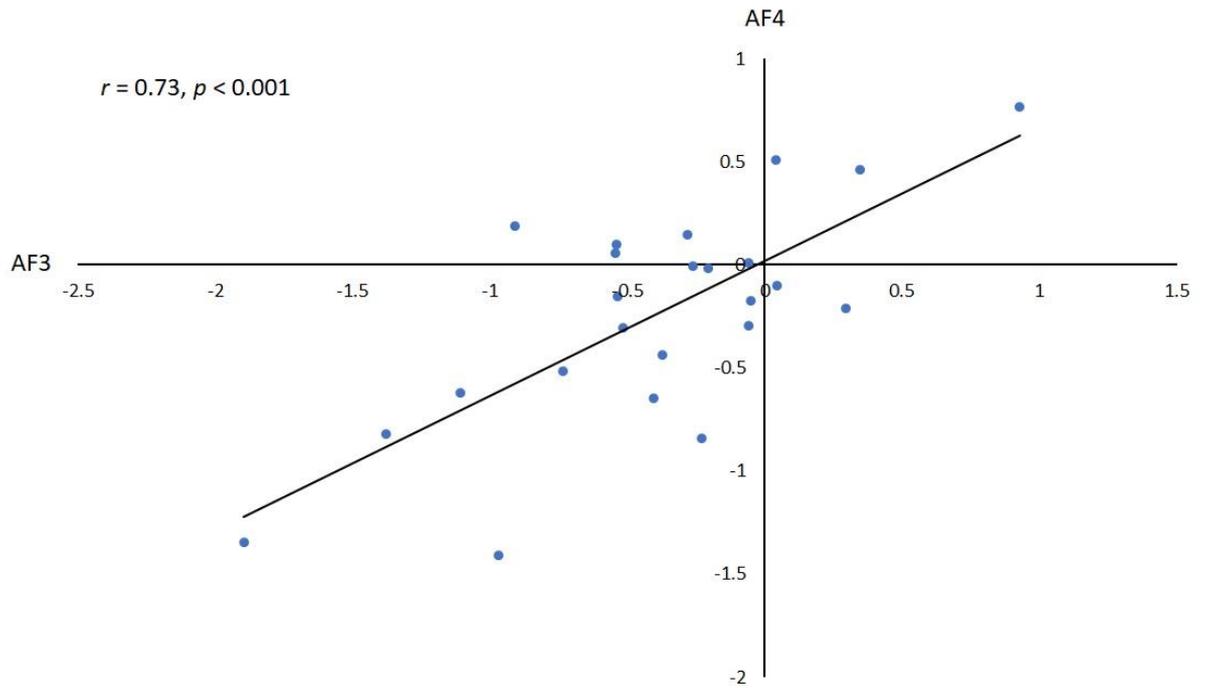


Figure 19: Relationship between the AF3 and AF4 positions for [TSI] in congruent blocks during exercise.

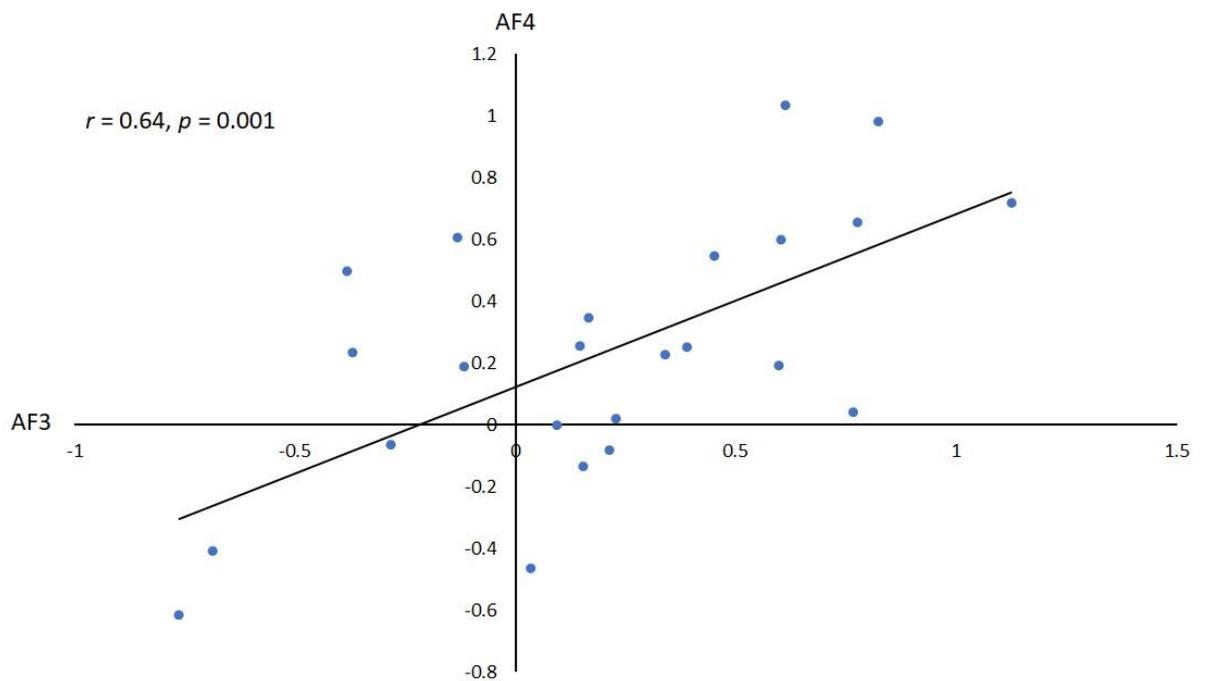


Figure 20: Relationship between the AF3 and AF4 positions for [HHb] in congruent blocks during exercise. *Note* Data presented in arbitrary units (A.U.)

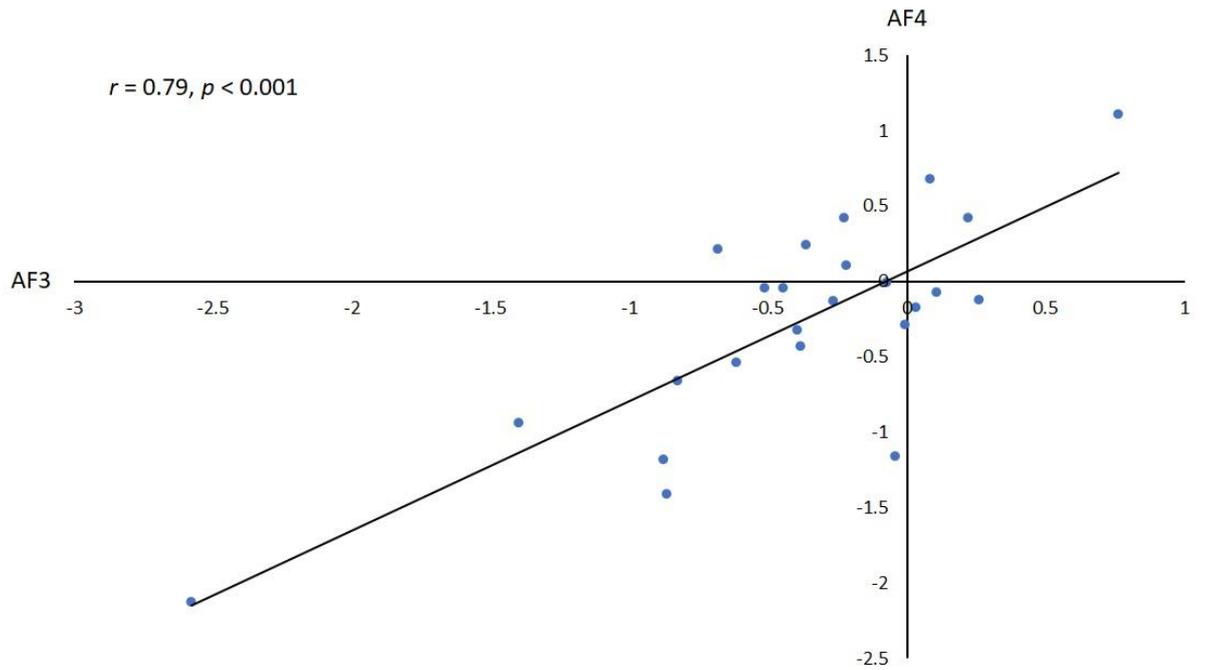


Figure 21: Relationship between the AF3 and AF4 positions for [Hbdiff] in congruent blocks during exercise. *Note* Data presented in arbitrary units (A.U.)

In the incongruent exercise trials there were also significant correlations between [TSI] values, [O<sub>2</sub>Hb] values, [HHb] values and [Hb<sub>diff</sub>] values in the AF3 and AF4 positions (see Table 24). In the incongruent exercise trials there were also significant correlations between [TSI] values, [O<sub>2</sub>Hb] values, [HHb] values and [Hb<sub>diff</sub>] values in the AF3 and AF4 positions (see Table 24). There were also significant correlations between [TSI] values in the AF3 position and [HHb] and [Hb<sub>diff</sub>] values in the AF4 position, between [O<sub>2</sub>Hb] values in the AF3 position and [TSI] and [Hb<sub>diff</sub>] values in the AF4 position and between [Hb<sub>diff</sub>] values in the AF3 position and [TSI] and [O<sub>2</sub>Hb] values in the AF4 position (see Table 24).

There were significant negative correlations between [tHb] values in the Fp1 position and [tHb] and [O<sub>2</sub>Hb] values in the Fp2 position and between [HHb] values in the Fp1 position and [tHb] values, [O<sub>2</sub>Hb] values and [Hb<sub>diff</sub>] values in the Fp2 position (see Table 23).

## 5.7 Discussion

This study aimed to determine whether the data collected using the Artinis Portalite NIRS device during a Stroop test correlated with data collected using an EEG. In addition, this study aimed to determine the optimum positioning for the NIRS optode to detect responses to the Stroop test. The principle original finding of this study was that the haemodynamic response to the Stroop task correlates with the electrical response detected by the EEG in the Delta and Theta frequency bands, indicating that the Artinis Portalite NIRS device may be a valid tool for cognitive research. These correlations were observed on both the left and right sides of the prefrontal cortex. Therefore, the hypothesis of the Artinis Portalite data correlating with the EEG signal can be partially accepted, however the right lateralisation of activation to the right side of the DLPFC cannot be accepted. An additional original finding of this study is that there is strong relationship between the haemodynamic response to the Stroop colour word task in the left and right sides of the DLPFC. This response is observed in both the Fp1 and Fp2 positions and the AF3 and AF4 positions but were more prevalent in the AF3 and AF4 positions. These correlations were particularly pronounced when the Stroop test was completed during exercise. This finding indicates that the haemodynamic response to a cognitive stimulus may not be lateralised to one hemisphere, but rather may be a whole structure response.

### 5.7.1 Lateralisation of cognitive responses

The DLPFC is consistently linked to responses to the Stroop colour word task, however, previous studies of the Stroop interference effect have been inconclusive in relation to the hemispheric response with some studies showing a left lateralisation of activation (Adleman et al., 2002; MacDonald, Cohen, Stenger & Carter, 2002; Stuss, Floden, Alexander, Levine & Katz, 2001; Yanagisawa et al., 2010) and others showing a right lateralisation (Vanderhasselt, De Raedt & Baeken, 2009; Vendrell et al., 1995). The inconsistency of findings from these studies could be a reflection of a range of different Stroop protocols used as different responses in the DLPFC have been observed in response to blocked or alternating congruent and incongruent trials, with more variability of responses observed in blocked conditions (Kane & Engle, 2002; Salo, Henik, & Robertson, 2001). However, the current findings indicate that the hemispheric response to the Stroop protocol may be linked providing an explanation for the inconsistency of findings.

Correlations were found between Stroop performance (RT and ACC) and EEG in all four positions and at different EEG frequencies which does not indicate one position or hemisphere for the localisation of the Stroop interference effect. However, correlations were found at the delta and theta frequencies with oxygen delivery [O<sub>2</sub>Hb], consumption [Hb<sub>diff</sub>] and blood flow [tHb] in the AF3 and AF4 positions. The link between haemodynamic response and the delta and theta frequencies of EEG has been previously demonstrated using BOLD fMRI (Michels et al., 2010) and the theta frequency has also been linked to increased working memory demands (Kwon et al., 2015) in frontal brain regions indicating this frequency is a useful measure of cognitive load. The lack of positional correlations with behavioural data does not rule out a role for the AF3 and AF4 positions in Stroop interference as correlations with behavioural data are not always a reflection of neurological activation (Jaeggi et al., 2003).

### **5.7.2 Bi-lateral hemispheric activation**

Connected activity has been noted in the prefrontal cortex between different ipsilateral regions (Cieslik et al., 2010) and interhemispheric interaction has been demonstrated between different neurological regions in response to a bimanual task (Fujiyama et al., 2016). A review of 3402 neuroimaging experiments has indicated that activation of a neurological region can be strongly connected to a response in the symmetrical region (Toro, Faux & Paus, 2008) but this co-activation is not generally reported in cognitive studies. Bilateral activation has been observed in the DLPFC in response to higher levels of task difficulty (Klingberg, O'Sullivan & Roland, 1997), which is at odds with the current findings as the greatest levels of coactivation occurred in response to the simpler (congruent) trials. A bi-lateral increase in [O<sub>2</sub>Hb] in frontal regions has been observed when a Stroop test was completed during exercise, although the localised specificity of this response was not detailed (Endo et al., 2013). These results were contradicted in another study which found no interaction between the left and right PFC regions (Bediz et al., 2016). It has been suggested that both hemispheres contribute to cognitive tasks by dividing the processes that need to be completed (Chiarello & Maxfield, 1996) but it is unclear whether this would produce the highly correlated responses observed in this study.

The correlations between the AF3 and AF4 positions were more pronounced during exercise which could mean that this linked response is caused by an underlying physiological mechanism. This explanation is in line with finding of a bi-lateral increase in [O<sub>2</sub>Hb] during a Stroop test completed during exercise by Endo and colleagues (2013).

Furthermore, it provides a potential explanation as to why this effect is not regularly detected in neuroimaging studies, as the robust nature of NIRS in relation to artefacts means that no restriction is placed on movement (Anderson et al., 2018; Dersosière et al., 2013; Ferrari et al., 2014; Tak & Ye, 2014), therefore physical activity related changes in response can be identified. However, a recent study conducted by Moi et al. (2019) which examined cerebral oxygenation responses to postural changes observed a substantial difference in TSI in the left and right frontal regions (measured as 2.5cm above the eyebrow) but no data was provided in relation to bi-lateral differences in other measures. The findings of Moi et al. (2019) indicate that the linked response may be caused by a bi-lateral activation in response to the Stroop protocol rather than a physiological response reflected by changes in blood flow. Furthermore, bi-lateral correlations were only found in the regions that were correlated with the EEG signal not in the Fp1 and Fp2 positions which reduces the likelihood of the response being due to extracerebral changes.

As the development of NIRS research is relatively new in comparison with other neuroimaging techniques, it would not be surprising to uncover neurophysiological responses not previously recognised. Although BOLD fMRI does examine haemodynamic responses it does so by examining changes in [HHb] (Kim & Bannettini, 2012; Logothetis & Pfeuffer, 2004) and does not provide indications of the other chromophore concentrations (e.g., [O<sub>2</sub>Hb]), or utilisation (e.g., [Hb<sub>diff</sub>]) which can be measured using NIRS (Perrey, 2008). In the current study the correlations between the right and left DLPFC were predominately reflected in changes in oxygen delivery ([O<sub>2</sub>Hb]) and oxygen consumption ([Hb<sub>diff</sub>], [TSI]) which would not be detected by BOLD fMRI studies.

### **5.7.3 Regional specificity and validation**

Unlike the previous chapter, the results of the current study do not provide conclusive support for the involvement of one specific prefrontal cortex location in Stroop interference. However, the observation of some correlations with EEG data indicates that the NIRS may be valid tool for detecting DLPFC activation. A correlation between the results obtained by NIRS and the results obtained using EEG demonstrates that the two techniques are likely to be measuring the same response and consequently the Artinis Portalite NIRS device may be valid tool for cognitive research (Campbell & Fiske, 1959). The correlations on the right side of the DLPFC (AF4 position) between the EEG and NIRS data were with the measures that are a stronger indication of neurological activation ([O<sub>2</sub>Hb], [Hb<sub>diff</sub>]) (Bhambhani et al., 2006; Ekkekakis, 2009; Gagnon et al., 2012; Perrey, 2008) than the

correlations on the left side (AF3) which were predominantly with [tHb] and [HHb] and may be more reflective of changes in blood flow rather than neurological activation (Hoshi & Tamura, 1993; Van Beekvelt et al., 2001). This may provide support for the role of a linked physiological mechanism rather than linked neurological activation and for the involvement of the AF4 region in completion of the Stroop task.

Although, the differences were not significant chromophore concentrations were larger in the AF4 than the AF3 position for all variables except [HHb]. This indicates higher activation within this region. The lack of significant differences was most likely caused by the large SD of the data set, which may have been influenced by interindividual differences in neurophysiological response (Cui et al., 2011; Huppert et al., 2006 Schecklmann et al., 2008). The links between the haemodynamic response on the right and left sides of the prefrontal cortex in the AF3 and AF4 positions, however, suggest that the lateralisation of the positioning when using NIRS is of less importance than ensuring that the NIRS probe is positioned over the correct region (Brodmann's area 9/46).

#### **5.7.4 Limitations**

There are limitations which must be addressed when considering the findings of the study. First is the issue of manual positioning of the NIRS device and the inherent risk of human error in the measurements as discussed in the previous chapter. This limitation is particularly relevant in this study as the NIRS devices were positioned using manual measurements following the modified 10-20 positioning system (Jasper, 1958), whereas the EEG electrodes were positioned using a pre-made electrode cap meaning that there is some risk that the two devices were not measuring exactly the same regions. A further limitation of this study is the blocked nature of the Stroop protocol. Whilst the blocked protocol was based on the design of a previous widely cited study (Milham et al., 2001) and used to better distinguish different levels of interference in response to varied task difficulty, there is evidence that this blocked design cause more variability in Stroop interference and therefore may have impacted the ability of this study to determine neurological responses (Kane & Engle, 2002; Salo, Henik, & Robertson, 2001). It must also be considered that only the frontal regions were examined due to the loss of signal quality when the NIRS is positioned over hair (Cui et al., 2011; Dersosière et al., 2013; Lloyd-Fox, Blasi & Elwell, 2010; Yücel et al., 2017).) and therefore any Stroop interference effect present in other regions may have been missed.

## 5.7 Conclusion

The findings of this study indicate that there is a link between activations observed using an EEG and the Artinis Portalite NIRS device. This demonstrates that the NIRS device may be a valid tool to detect changes in neurological activation in response to increased neurological demand and supports the potential usefulness of this tool to examine dual task interference in the prefrontal cortex. Whilst the findings of this study do not provide robust evidence for the validity of the Artinis NIRS device in detecting neurological activation they do provide initial evidence that the Artinis NIRS device may be a useful tool for inferring neurological activation from the haemodynamic response although further investigation of the mechanisms involved is required. Whilst conclusive support for the role of one side of the DLPFC in Stroop interference was not found, the right side appears to have a greater level of involvement than the left side. The linked haemodynamic response observed in this study provides initial evidence for bi-lateral activation in the prefrontal cortex and consequently the lateralisation of the signal position NIRS probe is likely to be less important than the regional location. Future research is required to further examine this effect and determine whether this response is reproducible.

As this chapter has indicated that the positions situated over the DLPFC (AF3 and AF4) are the regions involved in Stroop interference the next chapter will examine whether the results obtained in these regions are reliable both within and between days and determine whether the interhemispheric interaction in haemodynamic response can be reproduced during single trials within the same day and across multiple days.

## **Chapter 6: Between and within day reliability of cerebral oxygenation assessment using the Artinis Portalite NIRS device**

### **6.1 Introduction**

The previous chapter demonstrated that the Artinis Portalite NIRS device may be a valid tool for assessing neurological responses to the Stroop colour word task by demonstrating that the localised activity detected by the EEG correlates with activity detected by the NIRS device in the Delta and Theta frequency bands. In order to be confident that the Artinis Portalite NIRS device is accurately able to detect prefrontal cortex responses to dual task protocols it is important to establish the sensitivity of the NIRS device to detect neurological responses both to different tasks presented within the same day as well as to tasks presented on different days. This chapter will compare the within day and between day reliability of NIRS measurements in the DLPFC (AF3 and AF4 positions) at rest and during exercise by examining the intraclass correlation coefficient (ICC) of chromophore levels during a Stroop colour-word task over three separate trials during a single session (within day reliability) and across three separate sessions (between day reliability). The ICC will be used to examine test-retest reliability in resting trials and during exercise. Individual trials will also be examined to determine whether the optimum positioning of the NIRS probe to detect responses to the Stroop colour word task can be identified. In addition, in order to follow up the unexpected findings of a link between the haemodynamic response in the left and right DLPFC observed in the previous chapter, the correlation between chromophore concentrations in the AF3 and AF4 positions will be examined.

#### **6.1.1 The importance of measurement reliability in research**

Reliability is an important component of research which can be influenced by the equipment used (Atkinson & Nevill, 2001). Errors or variations in the measurement may be caused by a number of factors including systematic bias (e.g., learning error) or by random error (e.g., biological variations) (Atkinson & Nevill, 1998). Systematic bias usually reflects the trend in measurements to be different in one direction (positive/negative) and is commonly caused by either a learning effect (Coldwells, Atkinson & Reilly, 1994) or by insufficient between test recovery periods (Atkinson & Nevill, 1998).

The random error of a measurement is most likely to be influenced by spontaneous fluctuations in physiological responses (Atkinson & Nevill, 1998) such as the resting state fluctuations in brain activity (Cordes et al., 2000; Cordes et al., 2001; Tamura, Hoshi & Okada, 1997). NIRS measurements have been shown to be influenced by physiological noise such as respiration, arterial pulse oscillations and blood pressure Mayer waves (Boas et al., 2004; Canning & Scheutz, 2013; Hoshi, 2003; Hu, Hong & Ge, 2013) and consequently establishing the level of random error in NIRS measurements is of vital importance. The reliability of a measurement is intricately linked to the validity, as a piece of equipment cannot be considered as valid if the data obtained is not reliable (Atkinson & Nevill, 1998). When talking about a piece of equipment such as the Artinis Portalite NIRS device we are interested in the test-retest reliability which reflects the variations in measurements recorded by an instrument (Koo & Li, 2016).

### **6.1.2 Reliability of NIRS in cognitive research**

The reliability of NIRS measurements when assessing neurological activation has been assessed by a number of studies (e.g., Plichta et al., 2007; Schecklmann et al., 2008; Strangman et al., 2006), although as with the validity measurements this research has predominantly focussed around the reliability of fNIRS measurements. There is some evidence that NIRS measurements are highly reproducible over multiple testing sessions (Plichta et al., 2007) and a review by Bhambini et al. (2006) highlighted excellent levels of between day reliability for measures of cerebral oxygenation and blood flow across a range of interventions with both healthy and clinical populations. There are some indications that reliability may be influenced by the chromophore being examined, with reliability for [tHb] and [O<sub>2</sub>Hb] generally rating as higher than reliability of [HHb] (Kono et al., 2007; Schecklmann et al., 2008; Zhang et al., 2011), although high level of reliability in [HHb] has also been reported (Strangman et al., 2006). The variations in reliability levels of the different chromophores indicates that individual chromophore reliability may be influenced by task type. Excellent reliability has also been observed for assessments of changes in cerebral blood flow (Van de Ven et al., 2001).

Whilst reliability of NIRS assessed changes in oxygenation has been demonstrated at the group level (Bhambini et al., 2006; Strangman et al., 2008), reliability at the individual subject level has yet to be established (Biallas et al., 2012; Kono et al., 2007; Plichta et al., 2006, 2007a; Schecklmann et al., 2008; Scholkmann et al., 2014). A high level of intertrial variability has been demonstrated (Hu, Hong & Ge, 2013), which may result from

alterations in probe placement, effort expended, experimental familiarity, movement artefacts or spontaneous physiological fluctuations (Hu, Hong & Ge, 2013; Schecklmann et al., 2008; Strangman, Boas & Sutton, 2002; Zhang et al., 2011).

### **6.1.3 Reliability of the Artinis Portalite NIRS device**

Between variability of the Artinis Portalite NIRS device has been established for assessing exercise induced changes in skeletal muscle blood flow and consumption, with intraclass correlation coefficient (ICC) values of 0.75-0.96 recorded, indicating a good to excellent level of reliability (Lucero et al., 2018). Between day reliability of the Artinis Portalite has also been established for assessing changes in lower limb blood flow (Stone et al., 2016), however, between day reliability for cerebral responses has yet to be established. The within day reliability of the Artinis Portalite NIRS device in relation to the monitoring of cerebral oxygenation has been examined by one recently published study (Moi et al., 2019) that investigated the reliability of the minimum, maximum and mean values of [O<sub>2</sub>Hb], [HHb] and TSI. This study found that the mean [O<sub>2</sub>Hb] and [HHb] values showed the most reliability in the initial stages of the trial (ICC > 0.75) (Moi et al., 2019). A high level of reliability was established for both early and late trial [O<sub>2</sub>Hb] and [HHb] minimum values (ICC > 0.75), although as minimum values are not generally reported in the literature in relation to cerebral oxygenation the relevance of these changes in cerebral perfusion has yet to be established. However, although this study examined cerebral oxygenation it was examined in response to postural change rather than in response to a cognitive stimulus and therefore the reliability of the Artinis Portalite in representing haemodynamic responses due to neurological activation has yet to be established.

### **6.1.4 Interhemispheric DLPFC haemodynamic relationships during neurological activation**

A surprising finding of the previous chapter was the correlations between the haemodynamic responses in the left and right sides of the DLPFC, as the majority of literature has indicated a right or left lateralisation in response to the Stroop protocol (Millham et al, 2001; Vanderhasselt, Raedt, Baeken, Leyman & D'haenen, 2006; Vendrell et al., 1995). A meta-analysis of neuroimaging studies indicated that co-activation of the symmetrical brain region does occur during cognitive tasks (Toro, Faux & Paus, 2008), however, this relationship is not widely reported. Bi-lateral activation in the prefrontal cortex has been noted in response to increased levels of task difficulty (Klingberg, O'Sullivan & Roland, 1997), and a bi-lateral increase in frontal [O<sub>2</sub>Hb] has been observed when a

Stroop test was completed during exercise, although the regional specificity of this response was not provided (Endo et al., 2013). This finding was contrasted in another study that found that there was no interaction between the left and right PFC regions (Bediz et al., 2016). Furthermore, a study examining haemodynamic response to postural changes has indicated that measures of oxygen consumption (TSI) were distinctly different in the left and right frontal regions (Moi et al., 2019). Although this study did not use a cognitive task, the different haemodynamics in the left and right frontal region indicates that the linked haemodynamic response observed in the previous chapter results from the cognitive task rather than as a result of a physiological mechanism. The correlations observed in the previous chapter were found when the response in three trials was averaged. NIRS measurements have previously been shown to have intertrial variability (Schecklmann et al., 2008) and this, coupled with the fact that activation in the DLPFC has been shown to have high levels of intertrial variability (Windischberger, Lamm, Bauer & Moser, 2002) makes it important to ascertain whether the linked haemodynamic response is observed at an individual trial level.

## 6.2 Aims

The aims of this study were twofold. The primary aim was to establish the within and between day reliability of the Artinis Portalite NIRS device for measuring the haemodynamic response to cognitive stimuli in the DLPFC (AF3 and AF4 positions). A secondary aim was established following the findings from the previous chapter and this was to determine whether the linked interhemispheric haemodynamic response observed in the previous chapter is also present at an individual trial level. The specific experimental questions for this chapter were:

1. Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements at rest on the same day?
2. Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements at rest on different days?
3. Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements during exercise on the same day?
4. Is the Artinis Portalite NIRS device reliable for assessing multiple cognitive measurements during exercise on different days?
5. Is there a correlation between haemodynamic response in the left and right prefrontal cortex during individual trials?

It was hypothesised that the Artinis Portalite would show acceptable reliability for measurements recorded in the same day and on different days. It was also hypothesised based on the results of the previous chapter that there would be a linked haemodynamic response between the left and right hemispheres.

## **6.3 Methods**

### **6.3.1 Participants**

Participant demographics, recruitment procedure, inclusion and exclusion criteria and pre session instructions were as outlined in section 5.3.1. Ethical approval was obtained from the University of Winchester ethics committee before the commencement of this study.

### **6.3.2 Sample size determination**

A sample size of 24 was determined to be sufficient to detect significant effects with power at the 0.80 level and an alpha of 0.05 as predicted by G\*power (Faul, Erdfelder, Lang & Buchner, 2007). The power calculation used the effect size of  $d = 0.70$  from the results of Ambrosini and Valessi (2017).

### **6.3.3 Experimental procedure**

Participants attended four testing sessions over a two-to-four week period with a minimum of 48 hours between sessions. All testing sessions were completed at the same time of day ( $\pm 2$  hours) and the same temperature controlled laboratory was used for all participants. Participants completed a graded exercise test, one session which consisted of four resting Stroop tests and four exercise Stroop tests and two sessions which consisted of one resting Stroop test and one exercise Stroop test. The longer session was always completed before the two shorter sessions.

### **6.3.4 Graded exercise test (GXT)**

During the initial testing session participants completed a GXT using a ramp protocol (see section 4.3.4 for a full explanation of the protocol). An electronically braked cycle ergometer (SRM Ergometer, Jülich, Germany) was used for completion of the test and breath by breath data was collected using an online gas analyser (Cortex Biophysik, Leipzig,

Germany). Participants commenced the test by cycling for five minutes with resistance set at 0 watts (W), which was used as a warm-up period. At the end of the five minute period the cycle ergometer increased the pedal resistance by 1 W every 3 seconds. Participants maintained a cadence of ~75 revolutions per minute (rpm) and continued cycling until they could no longer maintain this cadence. Heart rate was recorded using a chest strap and watch during the GXT (Polar Electro UK Ltd., Warwick, England) and maximum heart rate was used to verify maximum capacity had been reached.

### **6.3.5 Determination of Gas Exchange Threshold (GET)**

The gas exchange threshold (GET) was determined for each participant from a graph of the  $\text{VO}_2$  response. The GET was determined using the modified V-slope method (Beaver, Wassermann & Whipp, 1986; Davis, 1985) (see section 4.3.5) and independently verified by two researchers. Following determination of the GET a work rate equal to 90% GET was determined for each participant and this work rate was used as the exercise intensity during the exercise Stroop tests.

### **6.3.6 Stroop test protocol**

The Stroop test protocol was identical to that described in the previous chapter (see section 5.3.6) and consisted of one block of 36 congruent trials (e.g., the word red written in the colour red) followed by one block of 36 incongruent trials (e.g., the word red written in the colour blue) (see Figure 15).. A wireless keyboard with colour stickers affixed to the keys was used to collect responses to the Stroop protocol (see Figure 11). The Stroop test was presented on a Lenovo Ideapad 500 laptop with a 17" screen. The laptop was positioned 170 cm in front of participants during all trials and screen brightness was kept at a constant level throughout all sessions.

### **6.3.7. Experimental trials**

Each trial was initiated in the same manner. Participants were seated for five minutes whilst resting HR was taken and positioning measurements were made to affix the NIRS probes to the AF3 and AF4 positions using the modified 10-20 electrode positioning system (Jasper, 1958) (see Figure 12). Detailed measurements for locating the respective positions are outlined in appendix D. Bi-adhesive tape was used to create a strong connection between the NIRS probe and the skin in order to minimise movement artefacts created by the probe de-coupling from the head (Scheeren, Schober & Schwarte, 2012), and

extraneous light was minimised by covering the NIRS probes with a crepe bandage and black bandana (Canning & Scheutz, 2013; Hoshi et al., 2005). The lightweight battery pack for each NIRS probes was worn on an adjustable belt around the waist.

In the initial trial participants completed four resting Stroop tests and four exercise Stroop tests each separated by five minutes of rest (see Figure 16) Justification for the resting period is detailed in section 5.3.8. In the exercise Stroop tests participants completed five minutes of cycling at 90% GET before the commencement of the Stroop test. In order to complete the Stroop test participants assumed an upright seated position on the bike and the keyboard wireless keyboard was positioned in front of them by the researcher. Participants were instructed to maintain a cadence of  $\geq 70$  rpm throughout the bout of cycling exercise. During the resting period HR was monitored to ensure in returned to resting levels ( $\pm 10$  bpm) between trials.

In the second and third experimental trials procedure was as detailed above with the exception that participants only completed one resting and one exercise Stroop test.

### **6.3.8 NIRS Data collection**

Haemodynamic changes in response to the Stroop protocol were continuously monitored during the NIRS trials using the Artinis Portalite NIRS device (Artinis medical systems, Einsteinweg, The Netherlands). A full explanation of the NIRS device can be found in section 4.3.9.

### **6.3.9 Data analysis**

Data was smoothed using a Gaussian filter (via the Artinis Oxysoft software), following which mean values for  $[O_2Hb]$ ,  $[HHb]$ ,  $[tHb]$  and  $[TSI]$  were determined for each individual trial. A change in chromophore levels relative to a 10s baseline recorded prior to the start of each Stroop test was determined as described in chapter 4. Following this the relative values of  $[O_2Hb]$ ,  $[HHb]$  were used to calculate  $[Hb_{diff}]$  using the equation  $[Hb_{diff}] = ([O_2Hb] + [HHb])/2$ . The intraclass correlation coefficient (ICC) was used to determine the within and between day reliability of each chromophore concentration for congruent and incongruent trials. Based on the recommendations of Koo & Li (2016) both the ICC value and 95% confidence intervals were used to interpret the reliability. A Pearson's correlation was used to determine the relationships between chromophore levels in the AF3 and AF4 position for

each individual trial. Paired t-tests were used to assess the differences in individual chromophore concentrations in the AF3 and AF4 positions.

The alpha level for all data analysis was set at  $p < 0.05$ . Data was presented as mean  $\pm$  SD and 95% confidence intervals (95% CI) were also reported where appropriate. Effect sizes were interpreted as: small = 0.01, medium = 0.06, large = 0.14 according to guidelines from Cohen, Miles and Shevlin (2001).

## 6.4 Results

### 6.4.1 Within day reliability

#### *Resting trials*

Within day reliability in the AF4 position during congruent and incongruent blocks was moderate to good, with the ICC values representing a moderate to good level of reliability and the 95% confidence intervals representing a range between poor and good reliability (see Table 25). ICC values in the AF3 position were poor for all chromophores with the exception of [HHb] which represented a moderate level of reliability (see Table 25).

#### *Exercise trials*

Within day reliability in the AF3 position ICC values reflected a moderate level of reliability during congruent blocks with the 95% confidence intervals representing a range between poor and good reliability (see Table 26). In the AF4 position reliability was moderate to good during congruent blocks with significant ICC values for all chromophores. The 95% CI values represented a range between poor and good (see Table 26). During the incongruent blocks, reliability in the AF3 position was moderate to good and the ICC values were significant. The 95% confidence intervals represented a range between poor to moderate (see Table 26). In the AF4 position reliability in the incongruent blocks was moderate for [TSI] and [Hb<sub>diff</sub>] and poor for all other chromophores, 95% CI represented a range between poor and moderate reliability (see Table 26).

Table 25 Within day reliability for congruent and incongruent blocks during resting trials.

Position	[tHb]		[TSI]		[O <sub>2</sub> Hb]		[HHb]		[Hb <sub>diff</sub> ]	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
<i>Congruent</i>										
AF3	0.17	-0.64,0.61	0.41	-0.17,0.72	0.03	-1.03,0.52	0.66	0.32,0.84	0.03	-0.92,0.55
AF4	0.72 <sup>***</sup>	0.44,0.87	0.77 <sup>***</sup>	0.55,0.89	0.72 <sup>***</sup>	0.46,0.87	0.64 <sup>**</sup>	0.29,0.83	0.71 <sup>***</sup>	0.44,0.87
<i>Incongruent</i>										
AF3	0.38	-0.23,0.71	0.41	-0.17,0.73	0.36	0.26,0.70	0.54 <sup>*</sup>	0.08,0.78	0.41	-0.17,0.72
AF4	0.71 <sup>***</sup>	0.43,0.87	0.78 <sup>***</sup>	0.56,0.90	0.73 <sup>***</sup>	0.47,0.88	0.73 <sup>***</sup>	0.47,0.88	0.73 <sup>***</sup>	0.47,0.88

Note ICC = Intraclass correlation coefficient; 95% CI = 95% confidence intervals; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

Table 26 Within day reliability for congruent and incongruent blocks during exercise trials.

Position	[tHb]		[TSI]		[O <sub>2</sub> Hb]		[HHb]	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
<i>Congruent</i>								
AF3	0.63**	0.29,0.83	0.50*	0.02,0.77	0.57**	0.14,0.80	0.66**	0.33,0.84
AF4	0.71***	0.43,0.87	0.88***	0.37,0.85	0.70***	0.42,0.86	0.63**	0.28,0.83
<i>Incongruent</i>								
AF3	0.84***	0.69,0.93	0.70***	0.41,0.86	0.78***	0.57,0.90	0.76***	0.52,0.89
AF4	0.23	-0.52,0.64	0.71***	0.42,0.86	0.43	-0.13,0.73	-0.31	-1.58,0.39

Note ICC = Intraclass correlation coefficient; 95% CI = 95% confidence intervals; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

#### **6.4.2 Between day reliability**

##### *Resting trials*

Between day reliability in the congruent blocks was poor to moderate in the AF3 position with only [tHb] and [O<sub>2</sub>Hb] showing significant values for ICC. Values for 95% CI ranged from poor to moderate (see Table 27). In the AF4 position reliability was poor in congruent blocks for all chromophores (see Table 27). In the incongruent blocks the AF3 position showed poor to moderate reliability with ICC values showing significance for the [tHb] and [HHb] values. The AF4 position showed low to moderate reliability for incongruent blocks in the AF4 position with only [HHb] showing significant ICC values (see Table 27).

##### *Exercise trials*

Between day reliability in the congruent and incongruent blocks was poor to moderate in the AF3 position with significant ICC values for every chromophore. The 95% confidence interval values ranged from poor to moderate (see Table 28). In the AF4 position moderate reliability with significant ICC values was observed for [HHb] in the congruent and incongruent blocks. Poor reliability with significant ICC was observed for [TSI] in the incongruent blocks. For all other chromophores reliability was poor, and 95% confidence intervals ranged from poor to moderate (see Table 28).

Table 27 Between day reliability for congruent and incongruent blocks during resting trials.

Position	[tHb]		[TSI]		[O <sub>2</sub> Hb]		[HHb]		[Hb <sub>diff</sub> ]	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
<i>Congruent</i>										
AF3	0.54*	0.09,0.79	0.33	-0.31,0.69	0.49*	-0.01,0.76	0.53*	0.06,0.78	0.43	-0.12,0.74
AF4	0.01	-0.97,0.54	0.27	-0.43,0.60	0.09	-0.79,0.58	0.43	-0.12,0.74	0.29	-0.40,0.67
<i>Incongruent</i>										
AF3	0.47*	-0.04,0.76	0.10	-0.78,0.58	0.43	-0.13,0.73	0.51*	0.04,0.77	0.40	-0.19,0.72
AF4	-0.02	-1.01,0.53	0.26	-0.46,0.66	0.16	-0.65,0.61	0.52*	0.04,0.78	0.46*	-0.06,0.75

Note ICC = Intraclass correlation coefficient; 95% CI = 95% confidence intervals; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

Table 28 Between day reliability for congruent and incongruent blocks during exercise trials

Position	[tHb]		[TSI]		[O <sub>2</sub> Hb]		[HHb]		[Hb <sub>diff</sub> ]	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
<i>Congruent</i>										
AF3	0.70 <sup>***</sup>	0.40,0.86	0.67 <sup>**</sup>	0.35,0.85	0.76 <sup>***</sup>	0.54,0.89	0.44 <sup>*</sup>	-0.10,0.74	0.76 <sup>***</sup>	0.53,0.89
AF4	0.10	-0.77,0.58	0.17	-0.64,0.61	0.07	-0.96,0.54	0.50 <sup>*</sup>	0.01,0.77	0.06	-0.85,0.56
<i>Incongruent</i>										
AF3	0.44 <sup>*</sup>	-0.10,0.74	0.70 <sup>***</sup>	0.40,0.86	0.57 <sup>**</sup>	0.15,0.80	0.44 <sup>*</sup>	-0.10,0.74	0.67 <sup>**</sup>	0.35,0.85
AF4	-0.56	-2.07,0.28	0.49 <sup>*</sup>	0.00,0.76	-0.22	-1.40,0.44	0.63 <sup>**</sup>	0.27,0.83	0.28	-0.41,0.67

Note ICC = Intraclass correlation coefficient; 95% CI = 95% confidence intervals; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

### 6.4.3 Differences between AF3 and AF4 positions

#### *Congruent blocks*

Values for [Hb<sub>diff</sub>] were significantly higher in the AF4 position than the AF3 position in the session 1 (S1) trial 2 (T2), session 2 (S2) and session 3 (S3) resting trials (see Table 29) and in the S2 exercise trial (see Table 30). In S3 [tHb], [TSI] and [O<sub>2</sub>Hb] values were significantly higher in the AF4 position during the resting trials and [TSI] was significantly higher in the exercise trial (see Tables 29 & 30). In S2 [HHb] was significantly lower in the AF4 position than the AF3 position during the resting trial (see Table 29). In the S1 trial 1 (T1) and S1 trial 3 (T3) resting trials and the S1 T1, S1 T2, S1 T3 and S3 exercise trials there were no significant differences between the AF3 and AF4 positions.

#### *Incongruent blocks*

In the S1 T2 resting trial values for [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] were significantly higher in the AF4 positions than the AF3 position. In the S1 T3 resting trial [Hb<sub>diff</sub>] was significantly higher in the AF4 position. In the S2 resting trial values were significantly higher in the AF4 position than the AF3 position for [TSI], [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] and significantly lower for [HHb]. In the S3 resting trial values were significantly higher in the AF4 position than the AF3 position for [tHb], [TSI], [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] and significantly lower for [HHb] (see Table 29). In the exercise trials [TSI] was significantly higher in the AF4 position than the AF3 position in S1 T2, and in S1 T3 [tHb] and [O<sub>2</sub>Hb] were significantly higher in the AF4 position. No significant differences were observed in any of the other trials (see Table 30).

Table 29 Chromophore concentrations in each resting trial for the AF3 and AF3 positions

Note values are mean ( $\pm$ SD) and presented in A.U; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ; a =

Session	AF3						
	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]	[tHb]	[TSI]
<i>Congruent</i>							
Session 1							
Trial 1	0.32(2.94)	0.42(1.08)	0.47(2.32)	-0.15(0.94)	0.31(0.99)	0.15(3.28)	0.42(0.96)
Trial 2	0.31(1.79)	0.02(0.90)	0.22(1.49)	0.10(0.58)	0.06(0.69)	1.18(1.61)	0.29(0.64)
Trial 3	0.44(1.95)	0.22(1.35)	0.50(1.94)	-0.06(0.68)	0.28(1.08)	1.50(1.64)	0.58(0.78)
Session 2	-0.07(1.27)	0.54(0.96)	0.35(1.39)	-0.43(0.68)** <sup>b</sup>	0.39(0.89)	-0.19(1.27)	0.84(0.70)
Session 3	-0.61(1.57)	0.54(0.79)	0.14(1.45)	-0.75(0.92)	0.44(0.93)	0.29(1.13)** <sup>a</sup>	1.06(0.69)** <sup>a</sup>
<i>Incongruent</i>							
Session 1							
Trial 1	0.36(4.05)	0.67(1.26)	0.76(3.19)	-0.40(1.20)	0.58(1.31)	0.42(4.01)	0.68(1.07)
Trial 2	0.55(1.64)	0.13(0.85)	0.52(1.53)	0.03(0.60)	0.24(0.83)	1.56(2.11)	0.46(0.84)
Trial 3	0.74(2.15)	0.59(1.17)	0.99(1.88)	-0.25(0.84)	0.62(0.99)	1.58(2.44)	0.64(0.85)
Session 2	-0.49(1.97)	0.90(1.06)	0.52(1.95)	-1.01(0.91)* <sup>b</sup>	0.76(1.16)	-0.46(1.76)	1.60(0.70)** <sup>a</sup>
Session 3	-1.38(2.63)	0.97(1.16)	0.16(2.65)	-1.55(1.22)* <sup>b</sup>	0.86(1.59)	0.27(1.85)* <sup>a</sup>	2.03(1.01)** <sup>a</sup>

significantly higher than AF3, b = significantly higher than

Table 30 Chromophore concentrations in each exercise trial for the AF3 and AF4 positions

Note values are mean ( $\pm$ SD) and presented in A.U; \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ; a =

Session	AF3					[tHb]	[TSI]
	[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]		
<i>Congruent</i>							
Session 1							
Trial 1	0.75(1.30)	-0.38(0.80)	-0.26(1.25)	0.33(0.61)	-0.30(0.73)	0.23(1.43)	-0.49(0.77)
Trial 2	-0.36(1.49)	-0.50(0.88)	-0.60(1.47)	0.24(0.49)	-0.42(0.80)	1.35(6.82)	-0.39(0.84)
Trial 3	-0.85(2.51)	-0.29(0.84)	-0.88(2.30)	0.03(0.76)	-0.45(1.17)	-0.40(2.09)	-0.32(0.88)
Session 2	0.09(1.71)	-0.35(0.92)	-0.13(1.50)	0.22(0.59)	-0.18(0.76)	0.78(2.07)	-0.13(0.96) <sup>*a</sup>
Session 3	-0.29(2.40)	-0.20(0.68)	0.34(1.86)	0.05(0.89)	-0.19(0.82)	1.28(2.07)	0.36(0.82)
<i>Incongruent</i>							
Session 1							
Trial 1	0.08(1.30)	-0.38(0.80)	-0.28(1.25)	0.33(0.61)	-0.30(0.73)	0.12(1.19)	-0.30(0.60)
Trial 2	-0.36(1.49)	-0.50(0.88) <sup>*a</sup>	-0.60(1.47)	0.24(0.49)	-0.42(0.80)	0.20(1.37)	-0.16(0.67)
Trial 3	-0.85(2.51)	-0.29(0.84)	-0.88(2.30)	0.03(0.76)	-0.45(1.17)	-0.52(2.00) <sup>*a</sup>	-0.25(0.76) <sup>*a</sup>
Session 2	0.09(1.71)	-0.35(0.92)	-0.13(1.50)	0.22(0.59)	-0.18(0.76)	0.53(1.65)	-0.11(0.75)
Session 3	-0.29(2.40)	-0.20(0.82)	-0.34(1.86)	0.05(0.89)	-0.19(0.82)	0.31(1.39)	-0.03(0.46)

significantly higher than AF3, b = significantly higher than

#### **6.4.4 Interhemispheric correlations**

In both the resting and exercise trials significant correlations between the AF3 and AF4 positions were found in individual trials within the same session and in different sessions. Correlations varied dependent on the trial number and whether the trial was a resting or exercise trial. The interhemispheric correlations for both resting and exercise congruent and incongruent trials are displayed in Tables 31-34.

Table 31 Correlations between the AF3 and AF4 positions in the congruent resting trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
<i>S1 Trial 1</i>						
	[tHb]	$r = 0.59 (0.002)$	$r = 0.29 (0.18)$	$r = 0.60 (0.002)$	$r = 0.36 (0.08)$	$r = 0.53 (0.01)$
	[TSI]	$r = 0.22 (0.31)$	$r = 0.70 (< 0.001)$	$r = 0.39 (0.06)$	$r = -0.30 (0.16)$	$r = 0.60 (0.002)$
AF4	[O <sub>2</sub> Hb]	$r = 0.53 (0.01)$	$r = 0.40 (0.05)$	$r = 0.58 (0.003)$	$r = 0.21 (0.33)$	$r = 0.58 (0.003)$
	[HHb]	$r = 0.54 (0.01)$	$r = -0.25 (0.23)$	$r = 0.39 (0.06)$	$r = 0.74 (< 0.001)$	$r = 0.11 (0.62)$
	[Hb <sub>diff</sub> ]	$r = 0.41 (0.05)$	$r = 0.52 (0.01)$	$r = 0.52 (0.01)$	$r = 0.00 (1.00)$	$r = 0.61 (0.002)$
<i>S1 Trial 2</i>						
	[tHb]	$r = -0.02 (0.93)$	$r = -0.23 (0.29)$	$r = -0.07 (0.76)$	$r = 0.11 (0.61)$	$r = -0.12 (0.58)$
	[TSI]	$r = 0.09 (0.69)$	$r = 0.41 (0.05)$	$r = 0.23 (0.28)$	$r = -0.33 (0.12)$	$r = 0.38 (0.06)$
AF4	[O <sub>2</sub> Hb]	$r = 0.05 (0.82)$	$r = -0.04 (0.86)$	$r = 0.06 (0.79)$	$r = 0.01 (0.98)$	$r = 0.06 (0.78)$
	[HHb]	$r = -0.17 (0.42)$	$r = -0.51 (0.01)$	$r = -0.31 (0.14)$	$r = 0.28 (0.19)$	$r = -0.46 (0.03)$
	[Hb <sub>diff</sub> ]	$r = 0.12 (0.59)$	$r = 0.17 (0.44)$	$r = 0.18 (0.40)$	$r = -0.11 (0.62)$	$r = 0.24 (0.26)$
<i>S1 Trial 3</i>						
	[tHb]	$r = -0.18 (0.41)$	$r = -0.39 (0.06)$	$r = -0.32 (0.13)$	$r = 0.42 (0.04)$	$r = -0.42 (0.04)$
	[TSI]	$r = -0.33 (0.12)$	$r = -0.06 (0.80)$	$r = -0.21 (0.32)$	$r = -0.33 (0.12)$	$r = -0.09 (0.68)$
AF4	[O <sub>2</sub> Hb]	$r = -0.22 (0.30)$	$r = -0.30 (0.16)$	$r = -0.28 (0.19)$	$r = 0.17 (0.42)$	$r = -0.31 (0.15)$
	[HHb]	$r = 0.04 (0.86)$	$r = 0.29 (0.18)$	$r = -0.16 (0.44)$	$r = 0.58 (0.003)$	$r = -0.33 (0.12)$
	[Hb <sub>diff</sub> ]	$r = -0.21 (0.32)$	$r = -0.13 (0.54)$	$r = -0.17 (0.42)$	$r = -0.12 (0.58)$	$r = -0.12 (0.58)$
<i>Session 2</i>						
	[tHb]	$r = 0.40 (0.05)$	$r = 0.47 (0.02)$	$r = 0.53 (0.01)$	$r = -0.32 (0.12)$	$r = 0.53 (0.01)$
	[TSI]	$r = 0.33 (0.12)$	$r = 0.67 (< 0.001)$	$r = 0.54 (0.01)$	$r = 0.50 (0.01)$	$r = 0.61 (0.001)$
AF4	[O <sub>2</sub> Hb]	$r = 0.46 (0.03)$	$r = 0.61 (0.002)$	$r = 0.64 (0.001)$	$r = 0.45 (0.03)$	$r = 0.67 (< 0.001)$
	[HHb]	$r = -0.12 (0.57)$	$r = -0.28 (0.19)$	$r = -0.24 (0.27)$	$r = 0.25 (0.24)$	$r = -0.28 (0.19)$
	[Hb <sub>diff</sub> ]	$r = 0.41 (0.05)$	$r = 0.59 (0.002)$	$r = 0.60 (0.002)$	$r = 0.46 (0.03)$	$r = 0.64 (0.001)$
<i>Session 3</i>						
	[tHb]	$r = -0.19 (0.37)$	$r = -0.01 (0.97)$	$r = -0.17 (0.42)$	$r = -0.06 (0.80)$	$r = -0.11 (0.62)$
	[TSI]	$r = 0.06 (0.77)$	$r = 0.63 (0.001)$	$r = 0.33 (0.11)$	$r = -0.42 (0.04)$	$r = 0.47 (0.02)$
AF4	[O <sub>2</sub> Hb]	$r = -0.08 (0.72)$	$r = 0.37 (0.08)$	$r = 0.15 (0.48)$	$r = -0.37 (0.07)$	$r = 0.30 (0.15)$
	[HHb]	$r = -0.17 (0.43)$	$r = -0.53 (0.01)$	$r = -0.46 (0.02)$	$r = 0.44 (0.03)$	$r = -0.58 (0.003)$
	[Hb <sub>diff</sub> ]	$r = 0.03 (0.89)$	$r = 0.53 (0.01)$	$r = 0.34 (0.10)$	$r = 0.49 (0.02)$	$r = 0.51 (0.01)$

Note Significance values ( $p$  values) displayed in parentheses following  $r$  values

Table 32 Correlations between the AF3 and AF4 positions in the incongruent resting trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
<i>S1 Trial 1</i>						
	[tHb]	$r = 0.68 (<0.001)$	$r = 0.34 (0.10)$	$r = 0.68 (<0.001)$	$r = 0.47 (0.02)$	$r = 0.61 (0.002)$
	[TSI]	$r = 0.22 (0.31)$	$r = 0.55 (0.006)$	$r = 0.34 (0.11)$	$r = -0.15 (0.47)$	$r = 0.48 (0.02)$
AF4	[O <sub>2</sub> Hb]	$r = 0.64 (0.001)$	$r = 0.42 (0.04)$	$r = 0.67 (<0.001)$	$r = 0.36 (0.09)$	$r = 0.66 (0.001)$
	[HHb]	$r = 0.50 (0.01)$	$r = -0.01 (0.95)$	$r = 0.41 (0.05)$	$r = 0.60 (0.002)$	$r = 0.23 (0.29)$
	[Hb <sub>diff</sub> ]	$r = 0.51 (0.01)$	$r = 0.47 (0.02)$	$r = 0.59 (0.002)$	$r = 0.16 (0.44)$	$r = 0.64 (0.001)$
<i>S1 Trial 2</i>						
	[tHb]	$r = 0.14 (0.53)$	$r = -0.05 (0.82)$	$r = 0.14 (0.51)$	$r = 0.01 (0.98)$	$r = 0.13 (0.55)$
	[TSI]	$r = 0.23 (0.27)$	$r = 0.43 (0.03)$	$r = 0.37 (0.07)$	$r = -0.32 (0.13)$	$r = 0.46 (0.02)$
AF4	[O <sub>2</sub> Hb]	$r = 0.28 (0.19)$	$r = 0.15 (0.49)$	$r = 0.33 (0.11)$	$r = -0.10 (0.64)$	$r = 0.35 (0.10)$
	[HHb]	$r = -0.28 (0.18)$	$r = 0.44 (0.03)$	$r = -0.39 (0.06)$	$r = 0.23 (0.28)$	$r = -0.44 (0.03)$
	[Hb <sub>diff</sub> ]	$r = 0.36 (0.08)$	$r = 0.32 (0.13)$	$r = 0.46 (0.02)$	$r = -0.18 (0.39)$	$r = 0.49 (0.01)$
<i>S1 Trial 3</i>						
	[tHb]	$r = 0.32 (0.13)$	$r = -0.13 (0.53)$	$r = -0.10 (0.63)$	$r = 0.59 (0.002)$	$r = -0.15 (0.48)$
	[TSI]	$r = 0.15 (0.49)$	$r = 0.22 (0.32)$	$r = 0.18 (0.41)$	$r = -0.02 (0.92)$	$r = 0.08 (0.41)$
AF4	[O <sub>2</sub> Hb]	$r = 0.37 (0.07)$	$r = -0.04 (0.85)$	$r = 0.20 (0.34)$	$r = 0.50 (0.01)$	$r = -0.02 (0.94)$
	[HHb]	$r = 0.04 (0.84)$	$r = -0.27 (0.21)$	$r = -0.17 (0.43)$	$r = 0.49 (0.01)$	$r = -0.37 (0.07)$
	[Hb <sub>diff</sub> ]	$r = 0.37 (0.08)$	$r = 0.08 (0.69)$	$r = 0.26 (0.17)$	$r = 0.28 (0.18)$	$r = 0.16 (0.46)$
<i>Session 2</i>						
	[tHb]	$r = 0.37 (0.08)$	$r = 0.09 (0.69)$	$r = 0.36 (0.09)$	$r = 0.03 (0.90)$	$r = 0.53 (0.01)$
	[TSI]	$r = 0.30 (0.15)$	$r = 0.32 (0.13)$	$r = 0.37 (0.08)$	$r = -0.13 (0.55)$	$r = 0.61 (0.001)$
AF4	[O <sub>2</sub> Hb]	$r = 0.43 (0.04)$	$r = 0.18 (0.39)$	$r = 0.48 (0.02)$	$r = -0.10 (0.65)$	$r = 0.67 (<0.001)$
	[HHb]	$r = -0.06 (0.80)$	$r = -0.16 (0.47)$	$r = -0.16 (0.47)$	$r = 0.22 (0.31)$	$r = -0.28 (0.19)$
	[Hb <sub>diff</sub> ]	$r = 0.38 (0.07)$	$r = 0.22 (0.29)$	$r = 0.47 (0.02)$	$r = -0.18 (0.39)$	$r = 0.64 (0.001)$
<i>Session 3</i>						
	[tHb]	$r = 0.06 (0.80)$	$r = 0.01 (0.96)$	$r = 0.002 (0.99)$	$r = 0.12 (0.59)$	$r = -0.04 (0.84)$
	[TSI]	$r = 0.17 (0.43)$	$r = 0.47 (0.02)$	$r = 0.34 (0.11)$	$r = -0.37 (0.08)$	$r = 0.42 (0.04)$
AF4	[O <sub>2</sub> Hb]	$r = 0.19 (0.37)$	$r = 0.36 (0.08)$	$r = 0.32 (0.13)$	$r = -0.27 (0.20)$	$r = 0.37 (0.08)$
	[HHb]	$r = -0.19 (0.37)$	$r = -0.50 (0.01)$	$r = -0.45 (0.03)$	$r = 0.55 (0.01)$	$r = -0.58 (0.003)$
	[Hb <sub>diff</sub> ]	$r = 0.24 (0.27)$	$r = 0.51 (0.01)$	$r = 0.45 (0.03)$	$r = -0.47 (0.02)$	$r = 0.55 (0.01)$

Note Significance values ( $p$  values) displayed in parentheses following  $r$  values

Table 33 Correlations between the AF3 and AF4 positions in the congruent exercise trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
<i>S1 Trial 1</i>						
	[tHb]	$r = 0.19 (0.38)$	$r = 0.24 (0.26)$	$r = 0.27 (0.20)$	$r = -0.15 (0.47)$	$r = -0.29 (0.16)$
	[TSI]	$r = 0.10 (0.64)$	$r = 0.72 (<0.001)$	$r = 0.37 (0.08)$	$r = -0.53 (0.01)$	$r = 0.53 (0.01)$
AF4	[O <sub>2</sub> Hb]	$r = 0.19 (0.37)$	$r = 0.45 (0.03)$	$r = 0.37 (0.07)$	$r = -0.35 (0.09)$	$r = 0.46 (0.02)$
	[HHb]	$r = -0.04 (0.85)$	$r = -0.63 (0.001)$	$r = -0.33 (0.12)$	$r = 0.60 (0.09)$	$r = -0.52 (0.01)$
	[Hb <sub>diff</sub> ]	$r = 0.18 (0.41)$	$r = 0.58 (0.003)$	$r = 0.42 (0.04)$	$r = -0.48 (0.02)$	$r = 0.56 (0.01)$
<i>S1 Trial 2</i>						
	[tHb]	$r = 0.46 (0.03)$	$r = 0.37 (0.07)$	$r = 0.51 (0.01)$	$r = -0.14 (0.50)$	$r = 0.51 (0.01)$
	[TSI]	$r = 0.24 (0.27)$	$r = 0.55 (0.01)$	$r = 0.38 (0.07)$	$r = -0.44 (0.03)$	$r = 0.48 (0.02)$
AF4	[O <sub>2</sub> Hb]	$r = 0.44 (0.03)$	$r = 0.46 (0.03)$	$r = 0.35 (0.01)$	$r = -0.24 (0.25)$	$r = 0.56 (0.004)$
	[HHb]	$r = -0.09 (0.68)$	$r = -0.37 (0.07)$	$r = -0.21 (0.33)$	$r = 0.36 (0.08)$	$r = -0.30 (0.15)$
	[Hb <sub>diff</sub> ]	$r = 0.41 (0.05)$	$r = 0.49 (0.02)$	$r = 0.51 (0.01)$	$r = -0.30 (0.15)$	$r = 0.56 (0.004)$
<i>S1 Trial 3</i>						
	[tHb]	$r = 0.87 (<0.001)$	$r = 0.66 (<0.001)$	$r = 0.88 (<0.001)$	$r = 0.19 (0.37)$	$r = 0.81 (<0.001)$
	[TSI]	$r = 0.33 (0.12)$	$r = 0.78 (<0.001)$	$r = 0.53 (0.01)$	$r = -0.53 (0.01)$	$r = 0.69 (<0.001)$
AF4	[O <sub>2</sub> Hb]	$r = 0.78 (<0.001)$	$r = 0.81 (<0.001)$	$r = 0.88 (<0.001)$	$r = -0.09 (0.68)$	$r = 0.89 (<0.001)$
	[HHb]	$r = 0.28 (0.18)$	$r = -0.39 (0.06)$	$r = 0.06 (0.08)$	$r = 0.77 (<0.001)$	$r = -0.19 (0.37)$
	[Hb <sub>diff</sub> ]	$r = 0.60 (0.002)$	$r = 0.86 (<0.001)$	$r = 0.77 (<0.001)$	$r = -0.34 (0.11)$	$r = 0.86 (<0.001)$
<i>Session 2</i>						
	[tHb]	$r = 0.38 (0.07)$	$r = 0.42 (0.04)$	$r = 0.47 (0.02)$	$r = -0.08 (0.70)$	$r = 0.50 (0.01)$
	[TSI]	$r = 0.42 (0.04)$	$r = 0.85 (<0.001)$	$r = 0.65 (0.001)$	$r = -0.42 (0.04)$	$r = 0.81 (<0.001)$
AF4	[O <sub>2</sub> Hb]	$r = 0.42 (0.04)$	$r = 0.61 (0.002)$	$r = 0.58 (0.003)$	$r = -0.25 (0.24)$	$r = 0.67 (<0.001)$
	[HHb]	$r = -0.15 (0.50)$	$r = -0.63 (0.001)$	$r = -0.37 (0.08)$	$r = 0.51 (0.01)$	$r = -0.57 (0.004)$
	[Hb <sub>diff</sub> ]	$r = 0.42 (0.04)$	$r = 0.73 (<0.001)$	$r = 0.62 (0.001)$	$r = -0.37 (0.07)$	$r = 0.76 (<0.001)$
<i>Session 3</i>						
	[tHb]	$r = 0.04 (0.84)$	$r = -0.16 (0.46)$	$r = -0.002 (0.99)$	$r = 0.12 (0.57)$	$r = -0.07 (0.75)$
	[TSI]	$r = -0.32 (0.12)$	$r = -0.03 (0.90)$	$r = -0.25 (0.23)$	$r = -0.35 (0.10)$	$r = -0.10 (0.65)$
AF4	[O <sub>2</sub> Hb]	$r = -0.13 (0.54)$	$r = -0.10 (0.64)$	$r = -0.12 (0.57)$	$r = -0.10 (0.64)$	$r = -0.08 (0.70)$
	[HHb]	$r = -0.49 (0.02)$	$r = -0.18 (0.39)$	$r = -0.33 (0.12)$	$r = 0.63 (0.001)$	$r = 0.30 (0.89)$
	[Hb <sub>diff</sub> ]	$r = -0.29 (0.17)$	$r = -0.03 (0.88)$	$r = -0.23 (0.29)$	$r = -0.31 (0.14)$	$r = -0.09 (0.68)$

Note Significance values ( $p$  values) displayed in parentheses following  $r$  values

Table 34 Correlations between the AF3 and AF4 positions in the incongruent exercise trials

		AF3				
		[tHb]	[TSI]	[O <sub>2</sub> Hb]	[HHb]	[Hb <sub>diff</sub> ]
<i>S1 Trial 1</i>						
	[tHb]	$r = 0.15 (0.48)$	$r = 0.14 (0.50)$	$r = 0.14 (0.52)$	$r = -0.07 (0.74)$	$r = -0.09 (0.66)$
	[TSI]	$r = -0.02 (0.64)$	$r = 0.69 (<0.001)$	$r = 0.22 (0.31)$	$r = -0.48 (0.02)$	$r = 0.42 (0.04)$
AF4	[O <sub>2</sub> Hb]	$r = 0.11 (0.60)$	$r = 0.39 (0.06)$	$r = 0.23 (0.27)$	$r = -0.21 (0.33)$	$r = 0.31 (0.14)$
	[HHb]	$r = -0.08 (0.71)$	$r = -0.56 (0.01)$	$r = -0.23 (0.29)$	$r = 0.64 (0.001)$	$r = -0.50 (0.01)$
	[Hb <sub>diff</sub> ]	$r = 0.07 (0.76)$	$r = 0.53 (0.01)$	$r = 0.28 (0.19)$	$r = -0.41 (0.05)$	$r = 0.45 (0.01)$
<i>S1 Trial 2</i>						
	[tHb]	$r = 0.18 (0.40)$	$r = 0.22 (0.31)$	$r = 0.22 (0.31)$	$r = -0.02 (0.93)$	$r = 0.23 (0.28)$
	[TSI]	$r = 0.18 (0.40)$	$r = 0.58 (0.003)$	$r = 0.32 (0.13)$	$r = -0.31 (0.15)$	$r = 0.45 (0.03)$
AF4	[O <sub>2</sub> Hb]	$r = 0.22 (0.30)$	$r = 0.28 (0.18)$	$r = 0.27 (0.19)$	$r = -0.05 (0.81)$	$r = 0.30 (0.15)$
	[HHb]	$r = 0.07 (0.75)$	$r = 0.03 (0.88)$	$r = 0.05 (0.80)$	$r = 0.07 (0.76)$	$r = 0.03 (0.88)$
	[Hb <sub>diff</sub> ]	$r = 0.29 (0.17)$	$r = 0.40 (0.06)$	$r = 0.37 (0.07)$	$r = -0.12 (0.59)$	$r = 0.43 (0.04)$
<i>S1 Trial 3</i>						
	[tHb]	$r = 0.77 (<0.001)$	$r = 0.55 (0.01)$	$r = 0.77 (<0.001)$	$r = 0.11 (0.62)$	$r = 0.67 (<0.001)$
	[TSI]	$r = 0.26 (0.21)$	$r = 0.74 (<0.001)$	$r = 0.50 (0.01)$	$r = -0.58 (0.003)$	$r = 0.66 (<0.001)$
AF4	[O <sub>2</sub> Hb]	$r = 0.70 (<0.001)$	$r = 0.76 (<0.001)$	$r = 0.81 (<0.001)$	$r = -0.21 (0.33)$	$r = 0.82 (<0.001)$
	[HHb]	$r = 0.22 (0.30)$	$r = -0.45 (0.03)$	$r = -0.05 (0.82)$	$r = 0.73 (<0.001)$	$r = -0.30 (0.15)$
	[Hb <sub>diff</sub> ]	$r = 0.53 (0.01)$	$r = 0.83 (<0.001)$	$r = 0.73 (<0.001)$	$r = -0.46 (0.03)$	$r = 0.82 (<0.001)$
<i>Session 2</i>						
	[tHb]	$r = -0.02 (0.62)$	$r = 0.15 (0.49)$	$r = -0.01 (0.98)$	$r = -0.05 (0.82)$	$r = 0.02 (0.94)$
	[TSI]	$r = 0.13 (0.56)$	$r = 0.83 (<0.001)$	$r = 0.35 (0.09)$	$r = -0.48 (0.02)$	$r = 0.61 (0.002)$
AF4	[O <sub>2</sub> Hb]	$r = 0.02 (0.94)$	$r = 0.48 (0.02)$	$r = 0.14 (0.50)$	$r = -0.30 (0.15)$	$r = 0.29 (0.16)$
	[HHb]	$r = -0.07 (0.73)$	$r = -0.58 (0.003)$	$r = -0.28 (0.18)$	$r = 0.46 (0.02)$	$r = -0.52 (0.01)$
	[Hb <sub>diff</sub> ]	$r = 0.05 (0.82)$	$r = 0.72 (<0.001)$	$r = 0.27 (0.21)$	$r = -0.49 (0.02)$	$r = 0.51 (0.01)$
<i>Session 3</i>						
	[tHb]	$r = -0.26 (0.23)$	$r = -0.30 (0.16)$	$r = -0.34 (0.11)$	$r = 0.001 (1.00)$	$r = -0.33 (0.12)$
	[TSI]	$r = -0.30 (0.16)$	$r = 0.21 (0.34)$	$r = -0.16 (0.46)$	$r = -0.37 (0.08)$	$r = 0.07 (0.73)$
AF4	[O <sub>2</sub> Hb]	$r = -0.35 (0.09)$	$r = -0.07 (0.75)$	$r = -0.32 (0.13)$	$r = -0.23 (0.29)$	$r = -0.16 (0.44)$
	[HHb]	$r = 0.29 (0.18)$	$r = -0.54 (0.01)$	$r = -0.003 (0.99)$	$r = 0.60 (0.002)$	$r = -0.37 (0.08)$
	[Hb <sub>diff</sub> ]	$r = -0.39 (0.06)$	$r = 0.12 (0.59)$	$r = -0.26 (0.21)$	$r = -0.39 (0.06)$	$r = -0.02 (0.94)$

Note Significance values ( $p$  values) displayed in parentheses following  $r$  values

## 6.5 Discussion

This study aimed to determine whether the Artinis Portalite was a reliable tool for determining haemodynamic changes both for repeated trials on the same day and on different days. This study also aimed to determine whether the linked bi-lateral haemodynamic response observed in the previous chapter could also be determined during individual trials rather than when trials were averaged. The principle finding of this study was that within day test-retest reliability for measures of cerebral oxygenation was acceptable for the Artinis Portalite device, however, between day findings were less reliable. This finding means that whilst the Artinis Portalite may provide a suitable tool for assessing multiple measurements within the same session, comparisons of results obtained in multiple sessions may not produce reliable results. Therefore, the hypothesis of acceptable reliability on between and within day measures must be rejected. This study also determined that when individual trials were examined it was possible to localise activity effects more specifically to the right side of the DLPFC (AF4). Furthermore, it was determined that the bi-lateral haemodynamic response observed in chapter five is most likely due to a linked physiological response. The hypothesis of a linked haemodynamic response can be accepted.

### 6.5.1 Within day reliability

In the resting trials a reasonable level of reliability was found in the AF4 position in both congruent and incongruent blocks with most ICC scores falling at the upper end of the moderate range or the lower end of the good range (ICC = 0.69-0.88). In the AF3 position values in the resting trials showed poorer reliability with the majority values falling between the upper end of the poor range or the lower end of the moderate range (ICC = 0.41-0.55). A similar level of reliability was also found in the congruent blocks during the exercise trials, however, in the incongruent blocks, reliability in the AF4 position reduced whereas reliability in the AF3 position was higher than in resting trials. These differences in reliability between the positions and between resting and exercise trials indicates that the results obtained using NIRS is subject to the effects of blood flow changes as previously demonstrated by Stone et al. (2016) and Moi et al. (2019) limiting the reliability of the device to determine cognitive changes during moderate intensity exercise.

In exercise trials only the congruent trials showed a high level of reliability in the AF4 position compared to both the congruent and incongruent trials at rest which can be

attributed to the effects of exercise combined with cognitive challenge. In the AF4 position chromophore levels related to neural activity such as [TSI], [O<sub>2</sub>Hb] and [Hb<sub>diff</sub>] during the Stroop test were higher in the AF4 position than in the AF3 position, indicating a right lateralisation of the Stroop interference effect (Ferrari, Mottola & Quaresima, 2004; McManus, Collision & Cooper, 2018; Tempest, Eston & Parfitt, 2014). The neural activation induced by congruent trials is considerably lower than incongruent trials (Duncan-Johnson & Kopell, 1981, Milham et al., 2001), suggesting that during the congruent exercise trials the NIRS is most likely measuring the prefrontal cortex changes in blood flow that occur in response to exercise (Robertson & Marino, 2016; Thomas & Stephane, 2008; Yanagisawa et al., 2010) rather than in response to the Stroop test.

In the incongruent trials, however, although the changes measured in the AF3 position are most likely due to exercise responses, the changes in the AF4 position reflect a combination of response to exercise and response to the Stroop task with this combined effect reducing the reliability in the incongruent trials. The higher reliability observed in the AF3 position during incongruent indicates that the NIRS is reliable when measuring blood flow changes during exercise during repeated trials within the same session. This is line with previous findings indicating the reliability of NIRS to measure changes in cerebral blood flow (Van de Ven et al., 2001). This is an important finding as changes in cerebral blood flow due to exercise have been shown to take between 2 and 8 minutes to return to baseline levels (Byun et al., 2014). The current study has demonstrated that a five minute break between trials appears to be sufficient for blood flow changes to stabilise during moderate intensity exercise.

### **6.5.2 Between day reliability**

Between day reliability in the AF3 position followed the same patterns as within day reliability, with good levels of reliability observed only in the incongruent exercise trials (ICC = 0.49-0.63). Between day reliability in the AF4 position was considerably lower than within day reliability. Previous research has mixed findings in relation to between day reliability, with studies showing both high (Plichta et al., 2007a) and low (Hu, Hong & Ge, 2013) between day reliability. This effect may be explained by the different NIRS devices used. Between day reliability is affected by a number of factors including the positioning of the probe (Hoshi, 2011; Orihuela-Espina et al., 2010; Plichta et al., 2007a,b). fNIRS devices use caps for optode positioning (Ferrari & Quaresima, 2012) which is more likely to ensure consistent positioning of the probe unlike the Artinis Portalite which is reliant on the

researcher to manually measure the position of the probe using the modified 10-20 measurement system (Jasper, 1958). As neural activation has a high level of regional specificity (Serrien, Ivry & Swinnen, 2006) slight deviations of measurement between sessions as well as individual fluctuations in physiological noise and different coupling between the head and the optode are likely to have a substantial effect on the reliability of results (Hu, Hong & Ge, 2013; Schecklmann et al., 2008; Strangman, Boas & Sutton, 2002; Zhang et al., 2011). The fact that the between day reliability was stronger in the AF3 position may indicate that the NIRS device has higher levels of between day reliability in terms of determining the changes in cerebral blood flow in response to sustained exercise but this cannot be confirmed by this study alone. The different responses in the AF3 congruent and incongruent exercise trials may reflect an initial rapid increase in cerebral blood flow at the onset of exercise (Timinkul et al., 2008) which is likely to be a less stable response.

### **6.5.3 Determination of optimal position**

In the previous chapter although results indicated a more prominent role for the right side of the prefrontal cortex (supporting the results found in chapter four), no significant differences were observed between the chromophore levels in the AF3 and AF4 positions. The chromophore levels in the previous chapter were examined based on an average result from the three trials. In this study the trials were examined individually, which yielded a number of significant differences between the AF3 and AF4 positions in both resting and exercise trials. Of note were the incongruent resting trials, where all but the first trial of the first session showed a significantly higher level of oxygen consumption in the AF4 position compared to the AF3 position as reflected by  $[Hb_{diff}]$  values.

Oxygen consumption has been indicated as a key marker for determining neural activity as it measures not only the supply of oxygen to the region but also the utilisation (consumption) of that oxygen (Bhambhani et al., 2006; Ekkekakis, 2009; Hoshi & Tamura, 1993). This response was particularly consistent in the resting incongruent condition. Based on the reliability values the resting incongruent response seems to be more indicative of neural activation rather than changes in blood flow which provides further support for the involvement of the right side of the prefrontal cortex. This finding is in line with previous studies that have indicated a role for the right side of the prefrontal cortex in the Stroop interference effect (Millham et al, 2001; Vendrell et al., 1995). Moreover, it is consistent with the evidence that the right side of the prefrontal cortex is activated when a task has

high attentional requirements (Casey et al., 1997; Knight, Grabowecky & Scabini, 1995; Rubia et al., 2003).

#### **6.5.4 Linked interhemispheric haemodynamic response**

The previous chapter produced surprising findings of a linked bi-lateral haemodynamic response which was further investigated on an individual trial level in this chapter. It was concluded in the previous chapter that this response was either due to linked activation or to a physiological change unrelated to the cognitive task, the findings of the previous chapter indicated that the former was more likely. The results of this chapter, however, demonstrate support for the second explanation. The largest number of correlations were observed in the initial trial of the session or in the final exercise trial. When a region of the brain is first activated there is often an overshoot in haemodynamic response caused by rapid vasodilation meaning that there can often be an increase in CBF above what is required for metabolism within the activated region (Paulson et al., 2010; Perrey, 2008). This overshoot could be reflected in increased blood flow throughout the entirety of the DLPFC rather than localised to the region of activation. Furthermore, exercise has been shown to have a strong influence on changes in CBF within the prefrontal cortex (Dietrich, 2006; Tempest, Eston & Parfitt, 2014) and responses may be slow to return to baseline (Byun et al., 2014). This relationship would provide explanation for the higher number of correlations in the exercise conditions, particularly in the third trial of the second session, and would also explain why the correlations in the previous chapter were strongest in the congruent condition. In the current study the congruent trial was always completed prior to the incongruent trial meaning that any 'overshoot' would be most likely to be reflected in the congruent trials and have stabilised by the time the incongruent trials were undertaken.

#### **6.5.5 Limitations**

As discussed in previous chapters this study is limited by the manual positioning of the NIRS device. This is a particular limitation when examining between day reliability as it cannot be guaranteed that the device was placed in the same position on each day. A further potential limitation not only of this study but to future studies using NIRS is the effects of exercise induced cerebral blood flow on the signal. Whilst participants were seated for five minutes prior to the start of the trial, they may have had elevated levels of cerebral blood flow due to travel to the laboratory which could have influenced the comparability of sessions. As the increase in blood can take up to eight minutes to return to resting levels

(Byun et al., 2014), future studies using NIRS should incorporate longer seated resting periods before the start of the experimental trial.

## 6.6 Conclusion

From the data presented in this study a number of specific conclusions can be drawn. First, the Artinis Portalite NIRS device shows a good level of within day reliability but not between day reliability. Therefore, it does not appear to be useful for comparing responses to a cognitive task between different sessions. Second, the confirmation of the role of the right prefrontal cortex (AF4) rather than the left prefrontal cortex (AF3) in response to the Stroop task when trials are examined on an individual level. This finding supports those of previous chapters and provides evidence for the role of this region in tasks which require attentional control. The final finding was the provision of further clarity regarding the linked interhemispheric haemodynamic response observed in the previous chapter. The results of this study indicate an underlying physiological mechanism rather than a linked neural activation as the most likely cause for the correlated interhemispheric response. This unexpected finding is particularly interesting for enhancing the understanding of the usefulness of NIRS to determine cerebral haemodynamic responses and warrants further investigation.

The previous three chapters have served to determine the validity and reliability of the Artinis Portalite for detecting neural activation changes in a task that is attentionally demanding. The findings of the previous chapters have clarified the best way of processing the data, established that data collected using the Artinis Portalite device correlates with some of the data collected using the more established neuroimaging technique of EEG which indicates that it may produce valid data to examine cerebral oxygenation changes and determined that there is a reasonable level of within day reliability. Furthermore, the importance of the right DLPFC in attentionally demanding tasks has been established. The first experimental chapter (chapter 3) showed that the dual task protocol used did not impair novel skill performance but neither did it improve it. A number of potential causes for this lack of effects were identified, including the infrequent presentation of the audio cue. This will be addressed in the next chapter which will examine the effects of three different dual tasks on the performance of a novel skill and the effects of training in two different dual task conditions on novel skill learning. As the findings in chapter 3 were unable to identify any neurological underpinnings of the different dual task effects, the Artinis Portalite will be used to determine neurological responses in the right DLPFC (AF4).

In the light of the results of the current chapter analysis will focus on comparing the neurological activation in response to different tasks presented within a single session.

## **Chapter 7: The influence of dual tasks on skill learning and performance and the effects on prefrontal cortex activation**

### **7.1 Introduction**

The initial empirical chapter of this thesis (chapter three) demonstrated that a simple audio-response task presented during a continuous movement skill does not impair or enhance novel skill performance, however, the use of pupil dilation data lacked the sensitivity to determine the psychophysiological responses to the dual task protocols. Chapters four, five and six examined the use of a single position NIRS probe to determine the haemodynamic responses to a cognitive task which can be used to infer neurological activation (Anderson et al., 2018; Brigadoi et al., 2014). These chapters determined that NIRS has good validity and within day reliability for determining cerebral oxygenation changes in the prefrontal cortex in response to task with a high attentional load. These responses were lateralised to the right prefrontal cortex indicating that the AF4 position was the most appropriate to use to examine haemodynamic changes in response to dual tasks which are attentionally demanding.

This chapter will refine the application of the simple audio task and examine the effects of this task on performance and learning of a novel continuous movement skill when compared to tasks with a similar modality (specifically a choice audio task and a clock task). Moreover, NIRS measurements will be taken to examine any changes that may occur in the prefrontal cortex in response to this audio task.

#### **7.1.1 Dual Task Performance**

Dual tasks are generally shown to impair performance of a novel skill and this response has been linked to the limited availability of cognitive resources (Rémy et al., 2010). There are a number of theories regarding the mechanisms by which DTI occurs which are discussed in section 2.3.2. The most common explanation of this interference refers to a bottleneck in response selection, suggesting that the human brain lacks the capacity to process responses to two separate stimuli concurrently (Pashler, 1994a,b; Rémy et al., 2010). According to the response selection bottleneck (RSB) hypothesis, the response to a second stimulus cannot commence until the processes involved in responding to the primary stimulus have been completed (Schumacher et al., 2001). It must be noted that this theory

suggests that interference occurs even when secondary tasks are simple, providing that the secondary task presents a choice of responses (Pashler, 1994a). Furthermore, the two tasks do not need to share response modalities for this interference to occur (Pellecchia, 2005). Based on this theory a very simple secondary task requiring no choice in the response should not interfere with primary task performance. There are some instances where DTI has not been shown to occur (e.g., Donohue et al., 2014; Ruthruff et al., 2003). This lack of DTI is thought to be due to the temporal overlap between the two tasks being large enough to allow the response to the first task to be completed before the second task requires a response (Huestegge & Koche, 2010). Alternatively, the presence of a relatively simple dual task, rather than interfering with the primary task performance, can create the perfect level of activation, motivation and concentration (Curran & Stokes, 2003).

There is limited evidence, however, that dual tasks, can enhance rather than impair novel skill performance. Hemond, Brown and Robertson (2010) demonstrated that performance of a motor sequencing task was impaired by simultaneously counting coloured cues but enhanced by learning a sequence of colour cues. Hemond, Brown and Robertson (2010) concluded from these results that the presence of a dual task will not always result in interference with the primary task, and it is the nature of the processes being engaged that determine how a secondary task affects performance. Based on the findings of this study they suggested that tasks which engage similar neurological processes to the primary task may enhance rather than impair performance due to the greater engagement of neural networks (Hemond, Brown & Robertson, 2010). This conclusion is contradictory to traditional thinking which has suggested that dual task interference is increased when the tasks compete for the same neural resources (Rémy et al., 2010).

### **7.1.2 Dual task learning**

Whilst the findings of a dual task enhancing skill performance reported by Hemond, Brown and Robertson (2010) are not commonly observed, a number of studies have demonstrated benefits to learning when a dual task is present during practice. Adding a degree of difficulty in practice tends to promote greater effort and is assumed to reach payoff in test conditions (Wulf & Shea, 2002). Chiou and Chang (2016) showed that training in a dual task condition improved learning of a bimanual coordination task and Roche et al. (2007) demonstrated that learning of a visuomotor task was improved during dual task conditions. Malone and Bastien (2010) investigated the effect of a dual task condition on split belt

walking and found that, even when initial performance of a novel motor skill was impaired, learning (as indicated by a delayed retention test) was enhanced.

A further study by Song and Bédard (2015) also found that dual task conditions did not impair learning, however, this was only observed when the dual task was also present at retention. Although interestingly, they observed that the dual task at retention did not need to be the same as the one that was used during practice (Song & Bédard, 2015). When multiple stimuli are presented at the same time, they can either be consolidated in the memory as separate processes or in association with each other (Cahill, McGaugh & Weinberger, 2001) which may explain why Song and Bédard (2015) found that a dual task was required in the retention test in order to demonstrate learning following dual task training. Moreover, when more than one stimulus is presented at once it can either facilitate or interfere with the processing of other stimuli (Cahill, McGaugh & Weinberger, 2001).

Improvements in learning under dual task conditions have been shown to occur even when the two tasks have distinctly different mechanisms or modalities (Brown & Bennett, 2001). However, it does appear that the facilitation of learning effects is task dependent, with not all secondary tasks eliciting the same response (Goh, Gordon, Sullivan & Winstein, 2014; Goh et al., 2012; Goh Lee & Fisher, 2013; Roche et al., 2007). A number of potential mechanisms for the enhanced skill learning in dual task conditions have been proposed. Learning in dual task conditions may serve to speed up automatization of the primary task (Clark, 2015; Doyon & Benali, 2005), cause a greater investment in cognitive encoding, or improve the speed of information processing (Kantak & Winstein, 2012). Alternatively, the secondary task may simply cause a more challenging training environment which has been shown to be beneficial for skill learning (Andrieux, Boutin & Thon, 2015; Schmidt & Bjork, 1992; Lee & Wishart, 2005). One explanation that has been proposed by two studies is that the presence of a dual task helps to aid attentional focus during training sessions by adding a level of complexity to an otherwise simple task (Roche et al., 2007; Chiou & Chang, 2016).

The cause of dual task enhanced learning was investigated by Goh and colleagues (2012) who compared the effect of a complex task (as posited by Roche et al., 2007) to a task using similar neurological processes as proposed by Hemond, Brown and Robertson (2010) in their study of dual task performance. Goh et al. (2012) used a choice audio-response task and a simple audio-response task applied at different movement phases of

a pursuit motor task. The choice-response task was thought to engage the same neural networks as movement planning and the simple-response task to engage the same networks as movement execution. Benefits to learning were observed when the choice task was presented before the start of the movement and when the simple task was presented during the execution of the movement leading Goh and colleagues (2012) to conclude that dual task benefits on motor learning were observed due to task similarity rather than task complexity.

The tasks used in the aforementioned studies are short duration discrete tasks involving minimal movements. If the goal is to understand motor skill learning in general it is important to study the acquisition and learning of more complex skills that at least initially pose greater challenges to the cognitive capacity of the learner (Wulf & Shea, 2002). It could be that learning of a complex continuous motor task would not show the same improvements in dual task conditions as are observed in the acquisition of more simple discrete skills (Wulf & Shea, 2002). It is therefore important to determine whether the same improvements to learning when training in dual task conditions occur in more complex continuous tasks.

### **7.1.3 Assessment of learning**

Learning can be assessed either by a retention test where the skill is performed in the same conditions that were used in training or a transfer test where the learnt skill is performed in a new context (Kantak & Winstein, 2012; Wulf, 2013). The effect of a dual task during the assessment of skill retention has shown contrasting effects. Naveh-Benjamin, Kilb and Fisher (2006) found that the presence of a dual task during a retention test impaired performance at retention, whereas Song and Bédard (2015) showed that the presence of a dual task aided skill retention, but only when participants had been trained in dual task conditions. This may be explained by the theory that learning is optimised when the processing requirements during practice match those required in the retention test (Lee & Wishart, 2005).

#### **7.1.4 Neurological activation in dual task conditions**

In the last decade there has been a great deal of interest in identifying regions of the brain responsible for DTI and understanding the neurological responses to the dual task experimental paradigm. Various regions of the brain have been identified as potential locations for the DTI effect including, the inferior and posterior parietal cortex (Rémy et al., 2010; Sigman & Deheane, 2008), the cerebellum (Sigman & Deheane, 2008; Wu et al., 2013), the premotor cortex (Wu et al., 2013) and the supplementary motor area (Wu et al., 2013). Other authors have failed to find one specific region responsible for DTI instead noting a general uprating in activation in all regions associated with the primary task alone (Van Impe et al., 2011).

The PFC has been indicated as playing a role in DTI by a number of studies. An increase in PFC activation has been observed in relation to an increase in secondary task difficulty (Mirelman et al., 2014) and a shift in activity to the PFC has also been shown following a period of dual task training. Increased activation has been particularly noted in the DLPFC in dual task conditions compared to single task conditions (D'Esposito et al., 1995). This activation has been predominately observed in the right side of the DLPFC (Corbetta, Miezin, Dobmeyer, Shulman & Peterson, 1991; Johannsen et al., 1997; Mandrick et al., 2013). A recent systematic review of the neural correlates of dual task performance found that whilst there was no conclusive support for the involvement of one specific region, the PFC was consistently associated with activity changes during dual task conditions (Leone et al., 2017). Studies attempting to identify the regions of the brain involved in DTI have used a range of different primary and secondary tasks, which may indicate that DTI does not occur in one region but rather that it is determined by activation of multiple regions at once in response to the need to complete two tasks with differing demands.

Goh, Lee and Fisher (2013) attempted to replicate enhanced learning in dual task conditions observed by Goh et al. (2012) using transcranial magnetic stimulation (TMS) over the dorsal premotor cortex (dPM) to replicate the performance benefits observed when a choice response task was presented during the preparation phase of the movement. Although no imaging was performed, this region was chosen as it is thought to play a significant role in movement planning and preparation (Pastor-Bernier, Tremblay & Cisek, 2012; Pearce & Moran, 2012; ) and was therefore the region that the authors hypothesised was responsible for the dual task related improvements previously observed. The findings

of this study were indicative of the involvement of this region in dual task related improvements in performance, however, the study focussed on long term benefits to learning rather than immediate performance benefits (Goh, Lee & Fisher, 2013) and therefore may simply be indicative in the role of the dPM in performance of the choice response task rather than the involvement of this area in the enhanced performances observed by previous authors in response to the presence of a dual task (Hemond, Brown & Robertson, 2010). The right side of the DLPFC is associated with tasks requiring a high level of attentional focus (De Joux, Russel & Helton, 2013; De Joux et al., 2017; Toichi et al., 2004) and has previously been associated with dual task effects. This region is therefore the most likely to show any changes in activity in response to dual task interventions. Furthermore, previous chapters have indicated a role of the right side of the DLPFC in response to a Stroop protocol and this task has been shown to have similar neurological demands to a dual task protocol (Hommel & Eglau, 2002).

#### **7.1.5 Skill learning and neuroplasticity**

The human brain has an inherent level of plasticity (termed neuroplasticity) which allows skill learning and adaptation to occur (Winstein et al., 2014). Neuroplasticity can be defined as “the sum of molecular, physiological, and structural changes that alter motor output for a given sensory input” (Zeiler and Krakauer, 2013, p.2). Learning related neuroplasticity is generally localised to the regions of the cerebral cortex that mediate the behaviour being learnt (Kleim et al., 2002; Steele & Penhune, 2010), and changes in the motor cortex tend to occur during the later phases of skill learning. Therefore, neuroplasticity in the brain may not necessarily be associated with learning dependent behaviour changes (Kleim, Hogg, VandenBerg, Cooper, Bruneau & Remple, 2004). Erikson et al. (2007) observed learning related changes in the brain following dual task training. They found that regions of the brain that were active before dual task training, showed decreased activity afterwards. Furthermore, a training related shift in the location of dual task activity was observed with an area of the DLPFC that was previously not active becoming active after dual task training (Erickson et al., 2007).

## **7.2 Aims**

This study aimed to determine whether a dual-task which is expected to activate similar neurological processes as the primary task could be used to facilitate novel skill performance and learning. To determine whether training in dual task conditions improves

novel skill learning and whether learning is dependent on task type, to identify whether retention of a learned skill is facilitated by the presence of a dual task at retention and to identify whether training in dual task conditions alters the haemodynamic response to neurological activation and whether this activation differs dependent on task type. The specific experimental questions for this study were:

1. Can a secondary audio response task presented during a continuous motor skill improve novel skill performance?
2. Will training in dual task conditions improve novel skill learning compared to training in a single task condition and is learning dependent on dual task type?
3. Does having a dual task present during retention aid performance of a learnt skill?
4. Do different dual task protocols effect the haemodynamic response during novel skill performance?

The final research question for this chapter was amended slightly from the original question outlined in the introduction of this thesis. As the NIRS was shown to have low reliability for between day measurements the between group measures were examined within the same retention session rather than between the retention tests as originally intended. Therefore, the amended research question was:

5. Is there an effect of training group on the haemodynamic response within immediate and delayed skill retention tests?

It was hypothesised that the simple audio task would improve novel skill performance and learning compared to single task conditions or other dual task conditions and that retention performance for participants training in dual task conditions would be enhanced by the presence of a dual task at retention. It was also hypothesised that there would be increased oxygenation in the prefrontal cortex in the dual task conditions than the single task condition and that the response would be greatest in the most complex dual task (clock condition).

## **4.3 Methods**

### **7.3.1 Participants**

Fifty participants (27 male, 23 female; age:  $22.50 \pm 5.63$  years) were recruited to take part in this study using convenience sampling in two different ways. Advertisements were placed around the university and participants who responded and met the inclusion criteria

were recruited. In addition, advertisements were placed via a university system which grants course credit to participants who take part in research projects. The right of participants who were recruited using this method to withdraw from the study was not affected and if they chose to withdraw after completing part of the study, they were still granted credit for the sessions they had completed. The inclusion and exclusion criteria were as follows:

*Inclusion criteria*

- Male or female
- Aged 18-55
- Able to attend for testing sessions at the university over a six-to-seven week period

*Exclusion criteria*

- Uncorrected vision
- Uncorrected hearing
- Injury to the arm or shoulder affecting movement
- Previous experience of playing the game used in the primary task

All participants received a participant information sheet (see appendix A) and provided written, informed consent to participate (see appendix B). Ethical approval was obtained from the University ethics committee before the commencement of the study.

### **7.3.2 Sample size determination**

A sample size of 45 participants was determined to be sufficient to detect significant effects with power at the 0.80 level and an alpha of 0.05 as predicted by G\*power (Faul, Erdfelder, Lang & Buchner, 2007). The power determination was based on the results of Chiou and Chang (2016), using the effect size of  $\eta^2 = 0.50$  detailed in the paper in relation to group differences in motor skill learning. Fifty participants were recruited to take part in this study, however, due to participant drop out the number of participants retained for analysis was 45.

### **7.3.3 Experimental Procedure**

Participants attended for testing on six occasions. During the first session participants completed a familiarisation where they received standardised instructions for how to operate the game (primary task) and undertook three trials to practice the primary task in

isolation followed by one trial in each of the three dual task conditions. Participants then completed three trials in each of the control condition and three trials in each of the dual task conditions which were recorded as baseline measurements. The second, third and fourth sessions took place within ten days of each other and occurred at least seven days after the baseline session. During the second and third sessions participants completed 18 training trials in their assigned dual task condition. During the fourth session participants completed six trials in the training condition followed by three trials in each of the different dual task conditions and three trials in the control condition which were recorded as an immediate retention test. The fifth and sixth sessions took place seven days and 28 days after the fourth session respectively. In the fifth and sixth sessions participants completed three trials in the control condition and three trials in each of the different dual task conditions which were recorded as delayed retention tests.

Throughout the study each set of three trials was separated by a five minute break to minimise fatigue and allow NIRS responses to return to resting levels. The dual task conditions in the baseline and retention tests were applied in a counter-balanced, randomised order.

#### **7.3.4 Primary task**

The novel task used in this study was a ten pin bowling game entitled 'Pin Rush' from the Xbox Kinect™ Sports Package (Microsoft, Redmond, Washington). The game was displayed on a Samsung television (2.05m x 1.52m) which was situated on a table at a height of 1.2 m from the floor. Participants stood two metres away from the screen and directly in front of the Kinect sensor, and once in position the Kinect tracking was calibrated to the participant to ensure maximum accuracy. The Kinect sensor was placed on a table 0.73 m off the floor and positioned below and in line with the centre of the screen. The protocol of the game is explained in detail in section 3.3.4.

Participants were able to view their top score for each session and most recent score at the end of each game as knowledge of results of performance is crucial to facilitating learning (Ericsson, Krampe & Tesch-Römer, 1993) but were not able to view scores from other participants. Participants viewed the game play from the perspective of the avatar and were instructed to use only their dominant hand (45 right-handed, 5 left handed) and to continue playing until the time ran out. Participants had no experience of playing the game used and minimal or no experience of using the Xbox Kinect system.

Other than basic instructions on how to operate the game which was provided in the first session participants were given no information on how to complete the task and other than the game score participants were given no feedback during the session.

### **7.3.5 Secondary tasks**

Four secondary task conditions were used in this study. In the control (C) condition participants completed three trials of the game with no secondary task. In the simple response condition (SR) participants were required to respond to an audio cue by saying 'now' each time the cue was detected. The audio cue was 750Hz and was set to play at a random point during a nine second block which then repeated after the cue had been played meaning that the participants would not be able to predict the timing of the next cue. In the choice response condition (CR) the participants were required to respond to audio cues of high and low pitch by saying 'high' when they heard the high pitch cue (1000Hz) and 'low' when they heard the low pitch cue (500 Hz). The audio cue was set to play at a random point during a nine second block and the pitch of the cue was also randomised. In the clock condition (CL) participants heard a time in hours and minutes (12 hour clock) and were instructed to say 'yes' if at the time stated the hands of the clock would be on the same side of the clock face and 'no' if at the time stated the hands of the clock would be on opposite sides of the clock face if a line was drawn down the middle between six and twelve (see Figure 22). The clock cues were set to be a set interval apart throughout the trial, but this interval was randomly selected for each individual trial and varied between two and eight seconds. The simple audio cue was in line with the task described in Goh et al. (2012) and the choice audio cue in line with that described in Goh et al. (2012) and Gabbett & Abernathy (2012). The clock task was designed to create a more complex task along the same stimulus and response modality.

Following the baseline measurements participants were randomly divided into groups with an equal number of participants in each group (n = 15) for the training trials. All participants trained in the C, CR or SR conditions dependent on their assigned group. All secondary tasks were designed in Psychopy (Pierce, 2007) and played through the standard speaker on a Lenovo Ideapad 500 laptop set at a volume of 50.

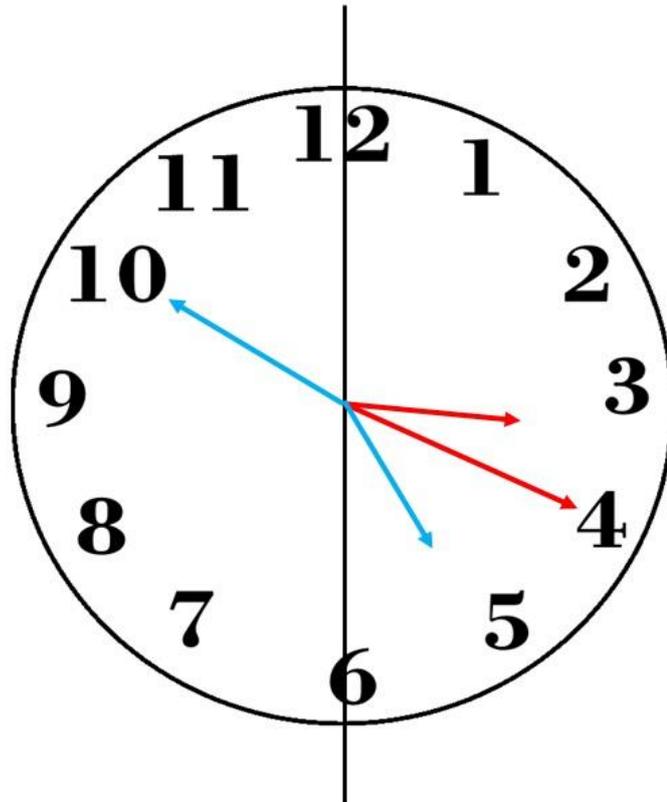


Figure 22: Visual representation of the clock task showing the line separating the two sides of the clock face. In this example the red hands (3.20) would represent a positive response (correct answer = yes) and the blue hands would represent a negative response (correct answer = no). Image obtained from <http://clipart-library.com> and adapted.

### 7.3.6 Measurements

Primary task performance was determined as game score (potential range: 0-700) in each trial and this was recorded manually by the researcher. The responses to the secondary tasks were recorded on an iPad recording application (TwistedWave voice recorder). Responses to the CR task and the CL task were analysed for accuracy. As no cues were missed during the SR task accuracy analysis was not completed for this task. Response times were not analysed as the responses in the CL condition were forced in some trials by short durations between cues and consequently it would not have been possible to compare response times between tasks.

### 7.3.7 NIRS data collection

Haemodynamic changes in response to the single and dual task conditions were continuously monitored using the Artinis Portalite NIRS device (Artinis medical systems, Einsteinweg, The Netherlands). A full explanation of the NIRS device can be found in section 4.3.9. The NIRS probe was positioned on the right side of the DLPFC with the midpoint of the probe situated over the AF4 position using the modified 10-20 positioning system (Jasper, 1958) (see Figure 12). Full details of the measurements used to position the probes are described in appendix D. The specifications of the Artinis Portalite device are detailed in section 4.3.9. A sampling rate of 50 Hz was used during all trials.

### 7.3.8 Data analysis

#### *Performance data*

Primary task performance within trial was averaged for each condition in the baseline and retention tests and conditions were compared using a 3 (group) x 4 (retention) x 4 (condition) mixed model ANOVA. Performance in the baseline and retention tests were also compared within condition using a 4 (trial) x 4 (condition) repeated measures ANOVA. Greenhouse-Geiser corrections were applied if sphericity was violated and Bonferroni post hoc comparisons were used to investigate significant differences. Separate one way ANOVAs were used to interrogate significant interaction effects.

#### *NIRS data analysis*

NIRS data was smoothed using a Gaussian filter (via the Artinis Oxysoft software), following which mean values for [O<sub>2</sub>Hb], [HHb], [tHb] and [TSI] were determined for each condition. A change in chromophore levels relative to a 10s baseline recorded prior to the start of each individual trial was determined as described in chapter 4. Following this the relative values of [O<sub>2</sub>Hb], [HHb] were used to calculate [Hb<sub>diff</sub>] using the equation  $[Hb_{diff}] = ([O_2Hb] + [HHb])/2$ . All chromophore values were averaged for each condition at baseline and during retention tests. The data for each chromophore was analysed using a 4 (trial) x 4 (condition) x 3 (group) repeated measures ANOVA. Due to the previous chapters findings of lack of reliability between days any significant differences were only considered if they occurred within session.

All data was analysed using SPSS version 25. The alpha level for all data analysis was set at  $p < 0.05$ . Data was presented as mean  $\pm$  SD and 95% confidence intervals (95% CI)

were also reported where appropriate. Effect sizes were interpreted as: small = 0.01, medium = 0.06, large = 0.14 according to guidelines from Cohen, Miles and Shevlin (2001).

## 7.4 Results

### 7.4.1 Baseline and retention test performance between conditions

When performance was compared across conditions at baseline and during retention tests a significant main effect for condition,  $F(3,132) = 4.70$ ,  $p = 0.004$ ,  $\eta_p^2 = 0.10$  and trial were observed,  $F(2.29,100.78) = 24.31$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.36$ . There were no significant Condition x Trial interaction effects,  $F(6.92,304.33) = 0.75$ ,  $p = 0.63$ ,  $\eta_p^2 = 0.02$ . Pairwise comparisons revealed that performance was significantly better in the SR condition than the CL condition ( $p = 0.02$ ) and significantly higher in the CR condition than the CL condition ( $p = 0.02$ ). Performance was also significantly better in all three retention tests than at baseline ( $p < 0.001$ ) (see Table 35).

### 7.4.2 Baseline and retention test performance between groups

The repeated measures ANOVA showed no main effect for Condition,  $F(3,126) = 0.40$ ,  $p = 0.75$ ,  $\eta_p^2 = 0.01$  or Group x Condition interaction,  $F(6,126) = 0.11$ ,  $p = 0.99$ ,  $\eta_p^2 = 0.01$ . A significant main effect was observed for Retention,  $F(1.59,66.63) = 22.24$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.35$  and a significant Retention x Group interaction,  $F(3.17,66.63) = 3.99$ ,  $p = 0.10$ ,  $\eta_p^2 = 0.16$ . There were no significant interaction effects for Condition x Retention,  $F(6.39,268.23) = 1.49$ ,  $p = 0.18$ ,  $\eta_p^2 = 0.03$  or Condition x Retention x Group,  $F(12.77,268.23) = 0.40$ ,  $p = 0.73$ ,  $\eta_p^2 = 0.03$ .

Pairwise comparisons of retention performance revealed that a greater improvement in performance was observed between Baseline and R1 than between R1 and R2 ( $p < 0.001$ ), between R2 and R3 ( $p = 0.004$ ) or between R1 and R3 ( $p = 0.01$ ). A larger improvement in performance was also observed between Baseline and R2 than between R1 and R2 ( $p < 0.001$ ), between R2 and R3 ( $p = 0.01$ ) or between R1 and R3 ( $p = 0.004$ ). Performance improvement between Baseline and R3 was also higher than between R1 and R2 ( $p < 0.001$ ), between R2 and R3 ( $p < 0.001$ ) or between R1 and R3 ( $p < 0.001$ ). It was also

observed that performance improved more between R1 and R3 than between R1 and R2 ( $p = 0.001$ ).

The Retention x Group interaction was investigated using a one way ANOVA which revealed a significant group difference at Baseline to R1,  $F(2,42) = 4.90, p = 0.01$  and at Baseline to R2,  $F(2,42) = 3.25, p = 0.05$ . With post hoc analysis revealing that performance improvement was significantly higher in the SR group than the C group at Baseline to R1 ( $p = 0.02$ ). At Baseline to R2 performance was higher in the SR group than the CR group although the Bonferroni corrected value was not significant ( $p = 0.08$ ).

(see Figure 23).

#### **7.4.3 Secondary task performance**

A repeated measures analysis of percentage accuracy in the clock task revealed no main effect for Trial,  $F(2.28,4.57) = 1.17, p = 0.32, \eta_p^2 = 0.03$  and no Trial x Group interaction effect,  $F(6,126) = 0.48, p = 0.82, \eta_p^2 = 0.02$ . In the choice response task there was also no main effect for Trial,  $F(3,126) = 1.20, p = 0.32, \eta_p^2 = 0.03$  and no Trial x Group interaction effect,  $F(6,126) = 0.62, p = 0.72, \eta_p^2 = 0.03$ .

Table 35 Primary task score in each of the four conditions at baseline and in retention tests

Condition	Baseline		R1		R2		R3	
	Mean( $\pm$ SD)	95% CI						
Control	118.01(59.29)	100.19,135.82	175.28(83.49)	150.20,200.37	164.67(80.49)	140.49,188.85	185.64(76.64)	162.62,206.67
SR	126.89(66.41)	106.94,146.84	173.08(82.94)	148.16,198.00	171.02(80.80)	146.75,195.30	183.02(84.30)	157.70,208.35
CR	121.47(60.71)	103.23,139.71	171.87(75.17)	149.29,194.46	168.73(77.99)	145.30,192.16	189.53(86.75)	163.47,215.60
CL	115.33(58.55)	97.74,132.92	162.04(72.04)	140.40,183.68	162.51(75.58)	139.80,185.22	168.50(71.87)	146.91,190.09

Note: R1 = retention 1, R2 = retention 2, R3 = retention 3

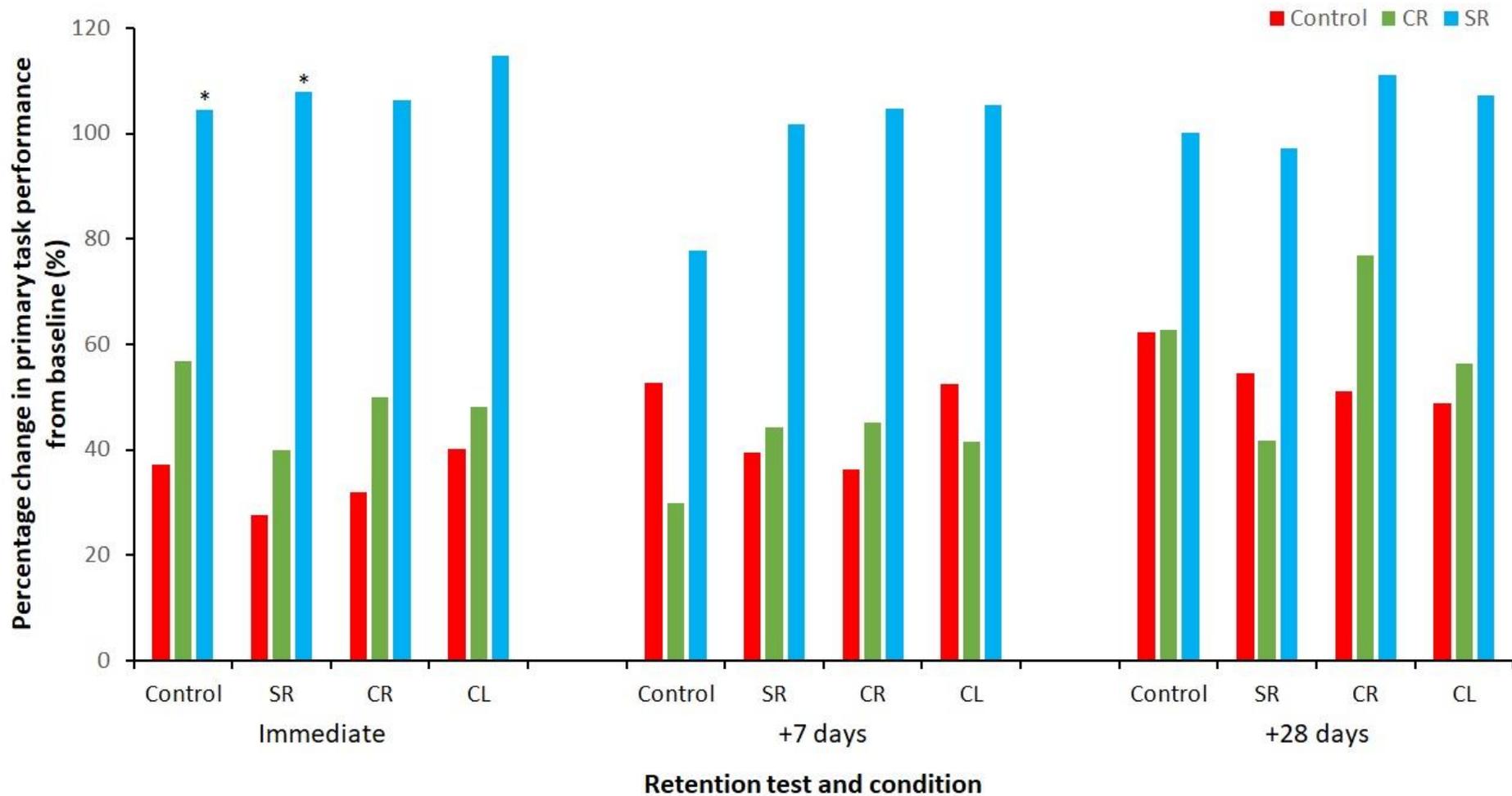


Figure 23: Percentage change from baseline in the three training groups at R1 (immediate), R2 (+7 days) and R3 (+28 days) in the four different conditions. *Note* Large variations in percentage change were observed so SD values were omitted from the graph for clarity, mean and SD performance values are displayed in Table 36, \* = significantly different from control ( $p < 0.05$ ).

Table 36 Primary task performance (game score) in baseline and retention tests for each group in the four conditions.

Group	Trial	Condition			
		Control	SR	CR	CL
Control	Baseline	122.35 (21.06)	133.42(28.94)	129.24(28.85)	120.36(22.17)
	R1	138.19(24.14)	145.2(27.03)	152.04(29.04)	135.62(29.25)
	R2	166.85(37.33)	154.82(26.70)	156.64(29.73)	151.49(32.03)
	R3	173.03(33.58)	177.64(40.26)	174.51(35.15)	149.38(30.58)
CR	Baseline	135.63(34.09)	143.76(30.25)	136.53(26.81)	133.24(27.29)
	R1	206.84(46.85)	190.36(45.85)	188.73(33.84)	177.93(36.49)
	R2	165.28(33.12)	184.51(33.61)	184.78(31.46)	172.24(36.40)
	R3	212.96(43.83)	195.44(35.91)	223.73(38.67)	191.69(33.76)
SR	Baseline	96.04(26.66)	103.49(19.38)	98.64(27.55)	92.40(22.01)
	R1	180.82(35.57)	183.69(40.41)	174.84(26.32)	172.56(27.24)
	R2	161.87(41.33)	173.73(36.65)	164.76(31.39)	163.80(37.02)
	R3	170.95(24.56)	175.98(29.16)	170.36(33.46)	164.42(24.86)

Note Values presented in mean ( $\pm$ SD)

#### 7.4.4 NIRS data

Values for [tHb] showed that there were no significant main effects for Trial,  $F(3,126) = 0.38, p = 0.77, \eta_p^2 = 0.01$  or Condition,  $F(2.34,98.36) = 0.28, p = 0.79, \eta_p^2 = 0.01$ . There were also no Trial x Group,  $F(6,126) = 1.75, p = 0.12, \eta_p^2 = 0.08$ , Condition x Group,  $F(4.68,98.36) = 1.38, p = 0.24, \eta_p^2 = 0.06$  or Trial x Condition,  $F(5.19,217.93) = 0.50, p = 0.79, \eta_p^2 = 0.01$  interaction effects.

Examination of [TSI] values revealed no main effects for Trial,  $F(3,126) = 2.46, p = 0.07, \eta_p^2 = 0.06$  or Condition,  $F(2.38,100.05) = 0.57, p = 0.60, \eta_p^2 = 0.01$ . There were also no interaction effects observed for Trial x Group,  $F(6,126) = 0.81, p = 0.57, \eta_p^2 = 0.04$ , Condition x Group,  $F(4.76,100.05) = 0.14, p = 0.98, \eta_p^2 = 0.01$  or Trial x Condition,  $F(5.54,,232.68) = 0.78, p = 0.58, \eta_p^2 = 0.02$ .

For [O<sub>2</sub>Hb] values there were no main effects observed for Trial,  $F(3,126) = 0.97, p = 0.41, \eta_p^2 = 0.02$  or Condition,  $F(2.33,97.94) = 0.26, p = 0.86, \eta_p^2 = 0.01$ . There were also no Trial x Group,  $F(6,126) = 1.79, p = 0.11, \eta_p^2 = 0.08$ , Condition x Group,  $F(4.66,97.94) = 1.11,$

$p = 0.36$ ,  $\eta_p^2 = 0.10$  or Trial x Condition,  $F(2.27, 229.54) = 0.42$ ,  $p = 0.93$ ,  $\eta_p^2 = 0.01$  interaction effects.

No main effects of Trial,  $F(2.01, 84.42) = 0.41$ ,  $p = 0.67$ ,  $\eta_p^2 = 0.01$  or Condition,  $F(3, 126) = 0.73$ ,  $p = 0.53$ ,  $\eta_p^2 = 0.02$  were observed for [HHb] values. There were also no interaction effects observed for Trial x Group,  $F(4.02, 84.42) = 1.20$ ,  $p = 0.32$ ,  $\eta_p^2 = 0.05$ , Condition x Group,  $F(3, 126) = 0.60$ ,  $p = 0.73$ ,  $\eta_p^2 = 0.03$  or Trial x Condition,  $F(6.87, 288.39) = 0.46$ ,  $p = 0.32$ ,  $\eta_p^2 = 0.01$ .

Values for [Hb<sub>diff</sub>] showed that there were no significant main effects for Trial,  $F(3, 126) = 1.68$ ,  $p = 0.17$ ,  $\eta_p^2 = 0.04$  or Condition,  $F(2.38, 99.86) = 0.35$ ,  $p = 0.74$ ,  $\eta_p^2 = 0.01$ . There were also no Trial x Group,  $F(6, 126) = 1.58$ ,  $p = 0.16$ ,  $\eta_p^2 = 0.07$ , Condition x Group,  $F(4.76, 99.86) = 0.72$ ,  $p = 0.64$ ,  $\eta_p^2 = 0.03$  or Trial x Condition,  $F(6.05, 254.29) = 0.36$ ,  $p = 0.61$ ,  $\eta_p^2 = 0.01$  interaction effects.

## 7.5 Discussion

This study aimed to determine whether dual tasks could be used to facilitate novel skill performance and learning, whether changes in learning were influenced by task type, to identify whether retention was improved by the presence of a dual task and to determine the haemodynamic response to training in dual task conditions. The primary finding of this study was that, as hypothesised, skill learning when training in the SR condition was enhanced compared to training in the control and CR conditions. This improved learning was demonstrated in immediate and delayed retention tests and also in immediate and delayed transfer tests. However, contrary to the hypotheses baseline performance was not improved, there was no advantage to the presence of a dual task at retention and there were no differences in haemodynamic response between the conditions.

### 7.5.1 Dual task performance at baseline

The lack of differences in baseline performance in this study are contrary to the literature base, as the DTI which is commonly reported in the literature in response to a dual task paradigm (Beurskens, Steinberg, Antoniewicz, Wolff & Granacher, 2016; Nijboer et al., 2013; Pashler, 1994a; Strobach, Frensch & Schubert, 2012), was not observed even in the more complex clock task. This finding could be explained by the lack of crossover in the response modalities of the primary and secondary tasks as the primary task required a motor response and the secondary task required a vocal response. However, a number of

studies have shown the DTI occurs even when response modalities are distinctly different (Huestegge & Koche, 2010; Luck, 1998; Marois & Ivanov, 2005; Pashler, 1994a). This was supported by the findings of chapter three of this thesis where the backwards counting task caused significant DTI. There are incidences where DTI has not been observed (Kane & Engle, 2002; Ruthruff et al., 2003) and this has been explained by a large gap between the two stimuli requiring responses (Huestegge & Koche, 2010) or by the secondary task creating an optimum level of concentration (Curran & Stokes, 2003). The first of these explanations would not explain the results found in this study as the use of a continuous primary task meant that overlap was constantly present. The second explanation has more merit, however, the secondary tasks used in this study were different in terms of complexity and therefore it is unlikely that they would all have elicited optimum concentration levels.

There is evidence that individuals may choose to alter their attentional load by taking small breaks from completing a continuous primary task in order to produce a response to the secondary task (Hiraga et al., 2009). Whether this would have been a possibility in the current study without a noticeable decrease in performance is unclear but it has been shown that when no instructions on task priority are given, task priorities may be varied to meet personal performance goals (Janssen & Brimby, 2010) so a manipulation of task priority by the participants presents a potential explanation for the findings of this study. An alternative explanation comes from the fact that when two tasks are relatively simple when they are performed in isolation, increases in effort in a dual task situation can counteract DTI effects (Tombu & Jolicoeur, 2003). As no measures of single task performance were taken for the secondary tasks this explanation cannot be ruled out. A final explanation for the findings of this study is that there was a level of automatising of the primary task. DTI effects have been shown to be reduced when the task is familiar (Beilock, Wieringa & Carr, 2002; Oberauer & Kliegel, 2004), and although the specific task used in this study was novel, the movement profile (bowling action) may have been familiar enough to participants that they were able to achieve a level of automatising.

In addition to the lack of interference effects there was also no benefit to performance. Hemond, Brown and Robertson (2010), showed that the presence of a secondary task that engaged similar neural networks to the primary task enhanced novel skill performance and Goh et al. (2012) proposed that the simple audio response task used in this study engaged similar neural networks to the execution of a movement. However, the findings of this study did not replicate the results of Hemond, Brown and Robertson

(2010), showing no performance benefits in the simple audio response condition. One potential explanation for the lack of performance benefits was the lack of trials used in the performance session. Although longer duration than the trials used by Hemond, Brown and Robertson (2010), this study utilised only three trials per condition compared to the fifty performance trials used by Hemond and colleagues. Furthermore, the experimental protocol used in the study by Hemond, Brown and Robertson (2010) involved 180 trials preceding the performance trials so their findings could represent a learning rather than a performance effect. The secondary task used in this study was proposed by Goh and colleagues (2012) to engage similar neural networks to movement execution, however, it was not as closely related to the primary task as the secondary task used by Hemond, Brown and Robertson (2010) and consequently may not have elicited the same benefits to novel skill performance.

### **7.5.2 Primary task learning**

All groups improved performance from baseline in both single and dual task conditions during immediate and delayed retention tests. However, training in the SR condition showed improved learning compared to training in the CR or control conditions which was reflected a larger percentage improvement in all retention and transfer tests. These improvements were particularly apparent at the immediate retention test, but more importantly participants who trained in the SR condition showed greater improvements in performance four weeks after the end of training in both retention and transfer tests. Ability to maintain performance after an extended period without training is an indicator of the robustness of learning (Kantak & Winstein, 2012). Furthermore, the consolidation of a learnt task in the motor memory becomes more stable over time (Krakauer & Shadmehr, 2006; Robertson, Pascual-Leone & Miall, 2004), and therefore performance at delayed retention tests is an indicator of the stability of motor skill learning.

The finding of improved learning in the SR condition compared to the control and CR conditions is in line with the work of Goh et al. (2012), who found that that motor learning was enhanced by a simple audio task presented during the execution of a movement but impaired when a choice reaction task was used. The findings of this study extend the work of Goh and colleagues (2012) who measured learning with an immediate retention test which may not be a true reflection of motor learning (Kantak & Winstein, 2012), whereas this study has shown that the improvements in performance are stable for at least four weeks after the end of training. Furthermore, this study has shown that the SR

group was able to successfully perform the learnt skill in different dual task conditions than those that were present during training. Song & Bédard (2015) showed that individuals who had trained in dual task conditions only exhibited performance gains when a dual task was present during the retention test which is not the case with the current findings as improved performance was observed in the single task (control condition) as well as the dual task conditions. Being able to perform a learnt skill in a different condition than that which was present during training is described in motor learning literature as a transfer test and is an indicator of the strength of the learning (Kantak & Winstein, 2012; Sattelmayer, Elsig, Hilfiker & Baer, 2016; Wulf, 2013). Consequently, the dual task used in this study appears to not only have improved the performance of the task being trained but also facilitated performance in other dual task conditions (Bherer et al., 2005). The relatively simple dual task used in the SR condition could have served to promote an external focus of attention which has been shown to be beneficial not just for skill learning but also for dual task performance (Goh et al., 2019; Wulf, 2013).

The current study also demonstrated a lack of differences between the retention tests indicating that there was minimal drop off in performance between the immediate and delayed retention tests. Learning is often inferred from a consistent change in performance with little variability (Magill & Anderson, 2014) and therefore this lack of change between retention tests indicates that learning has occurred. It must be noted however, that this lack of change was observed across groups and therefore may not necessarily provide supporting evidence to the benefits of dual task training.

Dual task training has been shown to be particularly beneficial in clinical populations such as in people recovering from stroke or suffering from Parkinson's disease (An et al, 2014; Choi, Lee & Lee, 2015; Fritz, Cheek & Nicholas-Larson, 2015; Kim et al., 2014; Plummer et al., 2014) and therefore the greater improvement in the SR condition compared to the CR condition could mean that this dual task would be more effective than other dual tasks in a clinical setting.

### **7.5.3 Haemodynamic response**

The NIRS data failed to clarify the mechanisms behind the improved learning in this study. No differences were found in haemodynamic response between the conditions at baseline or in any of the retention tests. The lack of difference in haemodynamic response between conditions could indicate a lack of sensitivity of the NIRS to dual task responses

which is unlikely as NIRS has previously been shown to be sensitive to changes in response to dual task conditions (Leone et al., 2017; McKendrick et al., 2014). A more likely explanation is that the haemodynamic responses to the difference tasks were not lateralised to the right DLPFC examined in this study. The right side of the PFC is indicated in tasks requiring sustained attention (De Joux, Russel & Helton, 2013; De Joux et al., 2017) and in dual task responses (Erikson et al., 2007; McKendrick et al., 2013; Mirelman et al., 2014), however, haemodynamic responses may have been occurring in other regions which were not detected.

#### **7.5.4 Limitations**

The primary limitation of the current study centres around the number of trials used. While the training trials were sufficient to induce learning effects, they were not sufficient to fully induce consistency of performance as indicated by the high standard deviations in the data in all conditions, however, the lack of difference between retention tests does indicate a certain level of consistency had been achieved. Although each trial of the primary task used in this game lasted over a minute and the tasks used by Goh et al. (2012) were less than ten seconds in duration the participants in that study received 432 practice trials compared to the 42 training trials used in the current study. Although the total practice time was roughly equivalent between the two studies, a continuous task is more complex than a simple discrete task and requires the performer to make more decisions during the performance of the skill making it more challenging (Wulf & Shea, 2002).

Another potential limitation may come from the size of the groups, although a power calculation was undertaken and group sizes were larger than those used in previous studies examining dual task learning (Goh et al., 2012; Roche et al., 2007), there is evidence that motor learning studies are traditionally underpowered to detect statistically significant changes (Lohse, Buchanan & Miller, 2016), which may have limited the scope of the current study to establish statistically significant differences between groups.

Finally, limitations in the collection of the NIRS data must be considered. Whilst the previous chapter established that the NIRS had acceptable reliability for within day measurements, reliability was not established to determine between group changes and previous research has indicated that reliability of between group measurements can be variable (Schecklmann et al., 2008; Strangman et al., 2008). Potential variations in the positioning of NIRS probes limit the reliability and comparability of NIRS measurements

between day and between groups (Canning & Scheutz, 2013; Plichta et al., 2007) which could have been reflected in the low number of differences detected in this study. Furthermore, although the right side of the prefrontal cortex has been indicated as a region of interest in tasks requiring sustained attention (De Joux, Russel & Helton, 2013; De Joux et al., 2017), the role of this region with regards to haemodynamic responses to the tasks used was not established and therefore changes in activation could have occurred in other regions and been missed.

## **7.6 Conclusion**

The findings of this study demonstrate that training in a dual task condition with a simple secondary task enhances learning compared to when training in a single task condition or an alternative dual task condition. Task performance in this study at baseline was unaffected by the dual task conditions, and it cannot be established whether this was due to a lack of differences in task demands or due to the small number of performance trials assessed. The findings of this study were unable to establish the neural mechanisms behind the improvements in skill learning as the NIRS data did not detect sufficient between group differences to draw conclusions.

This final experimental chapter has established that dual tasks can be beneficial for skill learning and the subsequent section will discuss the findings of this experimental chapter in relation to the findings of previous chapters and discuss potential directions for future research.

## **8. General Discussion**

This thesis addressed several aims which were outlined in chapter one. In order to address these aims two main objectives were identified, the first to investigate the use of dual tasks to enhance novel skill performance and learning and the second to examine the validity and reliability of a single position NIRS device in order to utilise this imaging technique to examine the haemodynamic responses to dual task protocols. This general discussion will expand on the discussions within each of the five empirical chapters to examine the combined findings of in relation to the research aims, evaluate the limitations of this body of work and put forward the future direction of research based on the findings of the five studies conducted.

### **8.1 Principle findings**

The principle original finding of this thesis was that a simple dual task protocol applied during the acquisition of a novel motor skill aids skill learning and retention for a least four weeks following the end of training. This thesis also demonstrated that the Artinis Portalite NIRS device may prove to be a valid tool for assessing the haemodynamic responses to a cognitive task although robust evidence to support this was not obtained. Furthermore, the Artinis Portalite NIRS device has acceptable within day reliability but between day reliability is limited meaning that it is most useful for comparing measurements within the same day. No evidence was found to support the benefits of a dual task protocol in novel skill performance. Findings of this thesis also indicate that training in a more challenging dual task condition creates higher cognitive demands and does not elicit any benefits to skill acquisition above those obtained training in a single task condition. Therefore, the benefits of dual tasks in enhancing skill acquisition appear to be task dependent.

### **8.2 Dual tasks and skill performance**

This thesis aimed to establish whether novel task performance could be enhanced by the presence of a dual task. It was hypothesised that the presence of a dual task which engaged similar neural networks to the primary task would facilitate the performance of the primary task. Dual task protocols when applied during novel skill performance usually create interference and impair performance of one or both tasks (Beurskens et al., 2016; Nijboer et al., 2013; Pashler, 1994a; Strobach, Frensch & Schubert, 2012). There are some

incidences where DTI does not occur (Donohue et al., 2015; Huestegge & Koche, 2010; Ruthruff et al., 2003), however, these are usually linked to levels of proficiency in the primary task as greater proficiency in the skill being performed reduces the DTI experienced (Beilock et al., 2002; Beilock, Weiranga & Carr, 2002; Janssen & Brumby, 2015). The work of Hemond, Brown and Robertson (2010) produced an unusual finding with regards to dual task performance as their results showed an improvement in novel skill performance in dual task conditions when two tasks which engaged similar neurological processes were performed concurrently. If this effect were transferable from the static discrete motor sequencing tasks used by Hemond, Brown and Robertson (2010) to a continuous motor skill involving physical movement it could prove to be a valuable tool, not only for the acquisition of skills within a healthy population but also in the rehabilitation of neurological conditions such as stroke and traumatic brain injury, where patients are often struggle to perform everyday skills (Khan, Baguley & Cameron, 2003; Raghaven, 2015; Sunderland, Walker & Walker, 2006).

The findings of this thesis were unable to support those of Hemond, Brown and Robertson (2010) as there was no improvement in dual task performance when a simple dual task was used during novel skill performance. The dual task that was used had been proposed by Goh et al. (2012) to engage similar neurological processes to the execution of a motor task and therefore was considered an appropriate task to use in order to attempt to replicate the findings of Hemond, Brown and Robertson (2010). Two different variations of this task with fixed and variable times between audio cues were used in chapter three and chapter seven respectively, however, whilst primary task performance was not impaired it was also not enhanced in either study. The failure of the studies in this thesis to replicate the previous findings may be explained by a closer examination of the protocol used. Although Hemond and colleagues (2010) reported their findings as improved novel skill performance in dual task conditions, the number of repetitions of the task that was used (432) suggest that the effects they observed reflected a response to learning in dual task conditions rather than performance in dual task conditions. This is an important distinction as conditions which impair novel skill performance have been shown to improve learning and therefore, long term performance gains (Malone & Bastien, 2010). The limitations of the protocol used by Hemond, Brown and Robertson (2010), combined with the findings of this thesis indicate that there is currently no evidence to support the feasibility of using a dual task protocol to enhance novel skill performance.

### 8.3 Dual tasks and skill learning

In addition to examining the effects dual task protocols on novel skill performance, this thesis aimed to examine the effects on novel skill learning. While the findings of this thesis were unable to support the role of dual tasks in improving novel skill performance, evidence was found to support the role of the dual task proposed by Goh et al. (2012) in enhancing novel skill learning. While some authors have suggested that performers benefit from a skill focussed environment when a task is novel (Rémy et al., 2010; Schaefer, 2014), there is evidentiary support for the role of a challenging practice environment in aiding the acquisition of motor skills (Kantak & Winstein, 2012). Moreover, several studies have shown benefits of the presence of a dual task during training on skill learning and retention (Chiou & Chang, 2016; Goh et al., 2012; Goh et al., 2013; Roche et al., 2007; Song & Bédard, 2015).

Several explanations for improved skill learning in dual task conditions have been proposed, including facilitating attention (Chiou & Chang, 2016; Roche et al., 2007), improving motivation (Song & Bédard, 2015), promoting implicit learning (Masters, 1992) and a greater availability of neural resources (Goh et al., 2012). The limited number of studies showing these performance gains in healthy populations make it difficult to identify the mechanisms involved. There are a larger number of studies showing enhanced learning in clinical populations such as stroke survivors (An et al, 2014; Choi, Lee & Lee, 2015; Kim et al., 2014; Plummer et al., 2014), but due to the variety of neurological impairments suffered by these populations (Wade & Hower, 1987), it is challenging to use the findings of these studies to elucidate the mechanisms involved.

An interesting finding of the study conducted in chapter seven was the enhanced learning in the SR dual task condition compared to the CR condition. This finding is in line with that of Goh et al. (2012) who found that a choice response task presented during the execution of a movement did not improve skill learning whereas a simple response task did. Goh et al. (2012) proposed that this difference could be attributed to the different neurological processes evoked by the two secondary tasks. Specifically, Goh et al. (2012) suggested that the choice response task activated a neurological response associated with preparation of movement and therefore enhances learning only when presented prior to the start of the movement phase, whereas the simple audio response task activated a neurological response associated with the execution of a movement and therefore enhances learning when presented during the execution phase of the movement.

An alternative explanation is that the simple audio response task, lacking the requirement for a response which is generally considered to be detrimental to performance (Pashler, 1994a), served to facilitate an external focus of attention. A large body of research exists to support the benefits of an external focus of attention in skill learning (see Wulf, 2013 for a review), however, the benefits of an external focus attention are specifically said to arise from a focus on the movement outcome (Wulf, 2013). In the case of an audio response task, however, the focus would be on the audio cue rather than on the effect of the movement per se and therefore the question arises as to whether this can be considered an external focus of attention. The work of Beilock, Wierenga and Carr (2002) uses a dual task paradigm to assess attentional focus indicating that a dual task is a useful tool to use to direct attention. Furthermore the use of a dual task protocol by Masters (1992) to promote implicit motor learning indicates that the use of dual task protocol prevents focus of attention on internal mechanisms of the movement, in the same way that an external focus does according to definitions utilised in the external focus of attention literature (Wulf, 2013). Similar findings emerge from the quiet eye literature where a focus on gaze behaviour is said to facilitate an external focus of attention (e.g. Moore et al., 2012; Vickers, 2009). Therefore, the benefits of the simple audio response task could be attributed to the benefits associated with an external focus of attention.

In addition to the aim of identifying whether the dual task aided skill acquisition, this thesis also aimed to identify whether the presence of a dual task during a skill retention test aided skill performance. Previous findings of Song & Bédard (2015) demonstrated that a dual task present during skill learning aided skill learning only when there was a dual task present during retention, even if the dual task differed from the primary task. The findings of this thesis were unable to support the work of Song & Bédard (2015) as both groups that trained in dual task conditions performed better in the single task condition at baseline. This finding demonstrates that the benefits of training in a dual task condition for enhancing novel skill learning are not dependent on the presence of a dual task at retention.

#### **8.4 The validity of the Artinis Portalite NIRS device for assessing haemodynamic responses to a cognitive stimulus**

One of the aims of the thesis outlined in the introduction was to explore the neurological processes underpinning the dual task benefits to performance and learning demonstrated by previous authors (Goh et al., 2012; Hemond, Brown & Robertson, 2010). The first

empirical study of this thesis used mobile eye trackers in an attempt to identify neurological responses using pupil dilation, however, this study was unable to find any differences in pupillary responses despite significant behavioural differences. Therefore, in subsequent chapters the Artinis Portalite NIRS device was examined in relation to detecting haemodynamic changes in the prefrontal cortex which is a region previously shown to be activated by dual task protocols (Leone et al., 2017). In order to establish this technique as suitable for assessing haemodynamic responses to dual task protocols it was first necessary to establish the validity of the Artinis Portalite.

The second and third empirical chapters (chapter 4 and chapter 5) examined both the appropriate method of processing data and validated the device against an EEG. Significant correlations were found between the NIRS data and the EEG data in the AF3 and AF4 regions which align with the left and right sides of the DLPFC respectively (Wang, Lu, Gu & Hu, 2018), this indicates that the Artinis Portalite device may be a valid tool for assessing neurological responses in the PFC. This finding extends the evidence regarding the accuracy of the Artinis Portalite which has previously been shown to be a valid tool for measuring changes in blood volume within the calf (Stone et al., 2016). Whilst this is the first time that the validity of this device has been examined for determining haemodynamic responses to cognitive stimuli, it is in line with previous research that has validated fNIRS for cognitive assessments against both fMRI and EEG (Butti et al., 2006; Huppert, et al., 2006) indicating that there is a relationship between the data acquired using the NIRS device and the data acquired using an EEG.

### **8.5 The reliability of the Artinis Portalite NIRS device for assessing haemodynamic responses to cognitive stimuli**

In addition to establishing the validity of a tool it is also crucial to identify whether the results are replicable (Atkinson & Nevill, 2008). Therefore, this thesis also aimed to establish the between and within day reliability of the Artinis Portalite NIRS device and these were examined in chapter six. The evidence in the literature is mixed regarding the reliability of NIRS devices, with some studies indicating excellent between day reliability (Kono et al., 2007; Plichta et al., 2007b) and others indicating that is reliability is reduced by factors such as different probe positioning and underlying physiological noise (Canning & Scheutz, 2013; Hu, Hong & Ge, 2013). Most studies have shown stronger with day reliability (Bhambhani et al., 2006; Zhang et al., 2011) which is supported by the finding in this thesis. The evidence presented in chapter six indicated that while the Artinis Portalite showed

good within day reliability, between day reliability was limited. Therefore, the within day comparisons of haemodynamic responses to are more likely to provide reliable information than comparing data between day or between groups.

### **8.6 The neurological responses to a dual task protocol**

A further aim of this thesis was to identify the neurological responses to dual task protocols that enhance skill learning. In order to address this aim, two different techniques for assessing neurophysiological responses to the dual task were explored. The initial study in this thesis (chapter three) used mobile eye trackers to examine the dual task dependent pupillary response by determining pupil dilation. Pupil dilation is indicative of mental effort and physiological arousal (Nassaret al., 2012; van der Wel & van Steenbergen, 2018), however, the pupillometry data failed to indicate any differences between conditions. The final empirical study (chapter seven) utilised NIRS to examine activation in the right side of the DLPFC, the cortical region which is associated with maintenance of attention (De Joux, Russel & Helton, 2013; De Joux et al., 2017).

The NIRS data collected during this study was unable to demonstrate any between group differences or between condition differences which may indicate that the dual task related activity was not localised to the right prefrontal region or indeed that the NIRS device lacked the sensitivity to detect the changes induced by the dual task protocols. Further investigation, potentially using alternative neuroimaging tools may be required to further elucidate the processes involved.

### **8.7 Optimum positioning of the NIRS optode and linked haemodynamic responses**

Findings of chapter four and chapter five indicated a role of the right side of the prefrontal cortex in response to Stroop interference which was confirmed by single trial analysis in chapter six. This finding is in line with previous studies that have indicated involvement of the right side of the PFC in mediating the Stroop interference effect (Millham et al, 2001; Vendrell et al., 1995). The Stroop test is indicated to activate the brain in a similar way to dual task protocols (Hommel & Eglau, 2002) and the right side of the PFC has been shown to be the region responsible for mediating activity in response to tasks requiring sustained attention, including dual tasks (Hommel & Eglau, 2002). The right DLPFC was therefore chosen to investigate the responses to the dual task protocols in chapter seven.

An unexpected finding in chapter five was that of the linked bilateral haemodynamic response in the right and left DLPFC. Neural activity is generally lateralised to one hemisphere (Bediz et al., 2016) and although there is limited evidence of bilateral activation (Klingberg, O'Sullivan & Roland, 1997; Toro, Faux & Paus, 2008), the linked haemodynamic response appears to be an original finding. There are two possible mechanisms by which this linked response may occur. First, this response could indicate an interhemispheric response to the Stroop protocol used in the study. A bilateral response to the Stroop protocol would provide explanation for the lack of agreement in the literature with regards to the lateralisation of the Stroop interference effect (Millham et al, 2001; Vanderhasselt, Raedt, Baeken, Leyman & D'haenen, 2006; Vendrell et al., 1995). A bilateral hemispheric response to the Stroop task has been previously demonstrated by one study (Endo et al., 2013), however, this finding is not in line with the literature base in this area. A second possible explanation for the linked response is that it reflects an underlying physiological response.

NIRS data has been shown to be affected by physiological noise, for example from blood pressure oscillations or changes in extracerebral blood flow (Hu, Hong & Ge, 2013; Selb et al., 2014) as well as by changes in blood flow as a result of physical activity (Byun et al., 2014) and therefore, the linked response could be indicative of changes in blood flow rather than bilateral activation. In chapter six the bilateral response was examined further on an individual trial level and the findings of this chapter indicated that the linked response was most likely due to a physiological mechanism. In chapter five the interhemispheric correlations were particularly prevalent in the congruent blocks which were always undertaken first, and when data was analysed on a single trial level the most interhemispheric correlations were observed in the initial trial of the session or in the exercise trials. An overshoot in the cerebral blood flow response has been observed at the onset of neurological activation (Paulson et al., 2010) and therefore it can be suggested that the linked bilateral response observed in chapters five and six is indicative of an initial overshoot in response at the onset of the Stroop task as observed in the congruent trials or increased cerebral blood flow in response to physical activity. This is an interesting finding and it indicates that in order to gain a true reflection of the haemodynamic response to a cognitive stimulus it may be necessary to eliminate initial trials, or the start of a trial from data analysis.

## 8.8 Limitations

In addition to the limitations considered in the empirical chapters it is important to consider the limitations of the thesis as a whole. The initial consideration is the variability in the learning effects observed. While percentage change from baseline was consistently higher in the SR group for all conditions and all retention intervals, this improvement was only significant at the immediate retention test. As the differences in percentage improvement were considerable, this lack of statistical significance must be attributed to the high variability in the level of improvement between participants. However, a large effect size was observed for the between groups comparison indicating that, despite the lack of statistical significance there is evidence to support the benefits of training in a dual task condition. One factor that may have contributed to the variability is the primary task that was chosen. Whilst the reliability of the Xbox Kinect sensor has been established (Yang et al., 2014), making it suitable for use in a research setting, the task was a game and therefore designed to be fun rather than challenging. It cannot therefore be ruled out that some improvement in performance from baseline came from fluctuating levels of difficulty in the game play.

The positioning of the NIRS device chosen for investigating the haemodynamic response to the dual task protocol in chapter seven must also be considered as a methodological limitation of this work. Whilst the right side of the prefrontal cortex was chosen based on the findings of the previous chapters and the link between the processes of dual task attention and the Stroop protocol (Hommel & Eglau, 2002), no pilot work was undertaken to determine whether this was a suitable location for determining responses to this task and therefore it must be considered that a more definitive haemodynamic response could have been observed in a different cortical region.

Limitations must also be considered in relation the NIRS device used in this thesis. First and foremost are the limitations inherent to the NIRS device which are discussed in detail in section 2.5.6. There are two limitations which are particularly applicable within the scope of this thesis. The first is the limitation related to the positioning of the probe. As this was done using manual measurements based on external cranial characteristics, there is no way to guarantee that the same neurological region was being sampled during each session or in different participants (Yücel et al., 2017). The second limitation which is particularly relevant is the filtering of movement artefacts. Whilst NIRS is more robust than other neuroimaging techniques to movement, the artefacts resulting from movements still affect the signal (Brigadoi et al., 2014). In this thesis a Gaussian filter built into the analysis

software was used to smooth the data in post-processing, however, visual inspection of the data after the application of the filter still revealed spikes in the NIRS signal, which appeared to be due to movement artefacts. It could be that small differences in response were lost in the signal to noise ratio which occurred due to movements inherent in the primary task and therefore it may be necessary to identify a more robust movement filter.

### **8.9 Directions for future research**

The findings of this thesis provide many possible directions for future research. One area that would be especially relevant to pursue is that of whether the simple dual task protocol which aided learning in the healthy population sampled in this thesis would also be beneficial in clinical populations. Training in dual task conditions has been demonstrated by several researchers to be beneficial in aiding rehabilitation post stroke (An et al, 2014; Choi, Lee & Lee, 2015; Kim et al., 2014; Plummer et al., 2014) and balance in elderly populations (Azadian et al., 2016; Gregory et al., 2016; Hiyamizu et al., 2012; Worden & Vallis, 2014, and these studies have all used traditional dual task protocols which impair skill performance for the training. The dual task protocol used in this thesis did not impair performance and enhanced learning above the levels achieved by an alternative dual task protocol. Therefore, this simple dual task may have additional benefits for rehabilitation than those already observed.

In terms of research in non-clinical populations there are also some areas for future research that can be identified. First, it would be relevant to examine whether in addition to aiding novel task learning whether this dual task can be used to aid improvement of already acquired skills in elite performers. In addition, although the findings of this thesis did not support the benefits of the dual task protocol on novel skill performance, there is some evidence to suggest that a simple dual task may facilitate the maintenance of an external focus of attention (Roche et al., 2007) which has been shown to be useful in maintaining performance under pressure (Moore, Vine, Cooke, Ring & Wilson, 2012; Wilson & Richards, 2011). Therefore, the effects of the SR protocol used in this thesis should be examined in relation to facilitating skill performance under pressure.

The findings of a linked bilateral haemodynamic response also merit further investigation. Future research in this area could examine whether a bilateral response is observed at rest and during exercise in the presence or absence of a cognitive stimulus. It

would also be useful to establish whether this interhemispheric response occurs during different cognitive tasks.

## 9. Conclusion

The empirical chapters in this thesis have resulted in several novel findings. The presence of a simple dual task during the acquisition phase of a novel task is beneficial to skill learning and retention, including performance in transfer tests. Conversely, the presence of a more complex dual task does not facilitate learning compared to training in a single task condition. Despite the observed benefits to skill learning, novel skill performance is not facilitated by a simple dual task. These findings have relevance for understanding the processes underpinning skill acquisition and have potentially important applications within the field of neurological rehabilitation.

This thesis has also demonstrated that the Artinis Portalite NIRS device may be a valid and reliable tool for assessing haemodynamic responses to a cognitive stimulus, and consequently for inferring neurological activation. This has important methodological implications, particularly within the field of sport and health science research where this device is used to determine muscle and cerebral responses to interventions. A linked bilateral haemodynamic response was observed during both resting and exercise trials which indicates that the mechanisms underpinning the cerebral haemodynamic response within the frontal regions is not yet fully understood. This linked response may serve to mask the true haemodynamic response to neurological activation, consequently further research is necessary to investigate the mechanisms underpinning this response.

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## **Appendices**

Appendix A – Informed consent forms

Appendix B – Participant information sheets

Appendix C – PAR-Q

Appendix D – Probe positioning instructions

Appendix E – Borg scale

Appendix F – Fatigue scale

Appendix G – SPSS Output

## **A - Informed consent forms**



Department of Sport and Exercise  
Faculty of Business, Law and Sport

### **INFORMED CONSENT**

**Project Title: An Exploration of the Effect of Dual-Task Protocols to Enhance Skill Performance in Healthy Adults**

**Researcher: Kirsty Brock**

The purpose of this study has been clearly explained to me and any risks involved in my participation have been made explicitly clear. All my questions about it have been satisfactorily answered. In addition, I agree that:

- Information I give will only be used for completion of an MPhil/PhD project in the Department of Sport Sciences, University of Winchester and publications resulting from the project.
- My identity in this study will remain anonymous.
- I have the right to withdraw any of my data. I am also free to withdraw from the study.
- My data will be stored securely. Only the researcher and PhD supervisors will have access to the data.
- After the 5 years the data will be destroyed.

**Date:**

**Signed:**

Participant:

Researcher:



Department of Sport Sciences  
Faculty of Business, Law and Sport

### **INFORMED CONSENT**

**Project Title: Reproducibility of single position near infra-red spectroscopy placement in Fp1 and AF3 positions to assess prefrontal cortex activation**

**Researcher: Kirsty Brock**

The purpose of this study has been clearly explained to me and any risks involved in my participation have been made explicitly clear. All my questions about it have been satisfactorily answered. In addition, I agree that:

- Information I give will only be used for completion of an MPhil/PhD project in the Department of Sport and Exercise, University of Winchester and publications resulting from the project.
- My identity in this study will remain anonymous.
- I have the right to withdraw any of my data. I am also free to withdraw from the study.
- My data will be stored securely. Only the researcher and PhD supervisors will have access to the data.
- After the 5 years the data will be destroyed.

**Date:**

**Signed:**

Participant:

Researcher:

Department of Sport, Exercise and Health  
Faculty of Business, Law and Sport

### **INFORMED CONSENT**

**Project Title: Reliability of single position Near Infrared Spectroscopy probes to determine changes in prefrontal cortex activation**

**Researcher: Kirsty Brock**

The purpose of this study has been clearly explained to me and any risks involved in my participation have been made explicitly clear. All my questions about it have been satisfactorily answered. In addition, I agree that:

- Information I give will only be used for completion of an MPhil/PhD project in the Department of Sport and Exercise, University of Winchester and publications resulting from the project.
- My identity in this study will remain anonymous.
- I have the right to withdraw any of my data. I am also free to withdraw from the study.
- My data will be stored securely. Only the researcher and PhD supervisors will have access to the data.
- After the 5 years the data will be destroyed.

**Date:**

**Signed**

Participant:

Researcher:



Department of Sport Sciences  
Faculty of Business, Law and Sport

### **INFORMED CONSENT**

#### **Project Title: Investigating the Impact of Dual-Tasks on Neurological Activation During Learning of a Novel Task**

**Researcher: Kirsty Brock**

The purpose of this study has been clearly explained to me and any risks involved in my participation have been made explicitly clear. All my questions about it have been satisfactorily answered. In addition, I agree that:

- Information I give will only be used for completion of an MPhil/PhD project in the Department of Sport Sciences, University of Winchester and publications resulting from the project.
- My identity in this study will remain anonymous.
- I have the right to withdraw any of my data. I am also free to withdraw from the study.
- My data will be stored securely. Only the researcher and PhD supervisors will have access to the data.
- After the 5 years the data will be destroyed.

**Date:**

**Signed:**

Participant:

Researcher:

## Appendix B – Participant information sheets

**An Exploration of the Effect of Dual-Task Protocols on Skill Performance in Healthy Adults**  
**Information Sheet for Participants**

Thank you for showing an interest in the project. Please read this information sheet carefully before deciding whether or not to participate. If you choose to participate, we thank you in advance for the time and effort you have decided to devote to our investigation. If you choose not to participate there will be no disadvantage to you of any kind and we thank you for considering taking part in this project.

**What is the aim of the project?**

This project is being undertaken as part of the requirements for an MPhil/PhD at the University of Winchester. The overall aim of this PhD is to investigate potential benefits of completing two tasks at the same time (dual-task) on (re) learning of upper limb movements in stroke survivors. The completion of two similar tasks at the same time has the potential to improve performance of the main task. The specific aim of this project is to identify the effects of completing two tasks (dual-task) at the same time on performance of an upper limb skill task in healthy adults. This project will provide interesting information regarding the effects of dual-task performance on skill performance and will inform the design of future projects working with stroke survivors.

**What types of participants are needed?**

We are looking to recruit males and females aged 25 - 50 who are in good health and have no restrictions in upper limb mobility or uncorrected sight/hearing problems. Participants should also not wear glasses or hard contact lenses (soft contact lenses are fine) and should not be familiar with the Xbox Kinect Sports mini game called 'Pin Rush'. Participants should be willing

to attend the University of Winchester for testing on 4 occasions which will last no more than 1 hour each time.

### **What will participants be asked to do?**

Participants will be asked to attend for testing on 4 occasions, the length of each visit will be no more than 1 hour. During each visit participants will complete several trials of a game on an Xbox Kinect computer system in which you move your arm to simulate a bowling task. Participants will be asked to complete this task under 3 different dual-task conditions which will be applied in a random order. Participants will be asked to complete the game in a control condition with no secondary task, in an audio response condition when they will hear a noise every 15 seconds through the whole game and will be asked to respond by saying 'now' when they hear it and a backwards counting condition where participants will be required to count backwards from 100 in 3s whilst playing the game.

**Visit 1:** The first visit will be a familiarisation session allowing participants to become familiar with the laboratory surroundings, with the game that will be used for the main tests and with the eye-tracking equipment. Participants will initially complete 3 trials of the game and then a further 3 trials of the game wearing the eye-tracking equipment. This will be followed by a further 3 trials in the audio response and backwards counting dual-task conditions.

**Visit 2-4:** On the next 3 visits participants will complete 3 blocks of 3 trials of the bowling game in each of the 3 dual-task conditions which will be administered in a random order. Participants will receive a 5 minute break between each block of 3 trials. Game performance (score) and dual-task performance (accuracy) will be recorded on all trials and participants will wear eye-trackers throughout. Each visit will be separated by at least 7 days to minimise learning from one visit to the next.

### **Can participants change their mind and withdraw from the project?**

If at any time you decide you no longer wish to participate in this project (for any reason) you may withdraw without disadvantage to yourself of any kind.

### **What data or information will be collected and what use will be made of it?**

Any data collected during this test will be used to establish profiles across the group of participants involved and these cumulative scores may be available for public inspection in research journals and/or at seminars or conferences. In addition, individual data/responses indicative of the typical response may also be presented. However, in all cases, anonymity will be strictly preserved. Participant codes will be used for all data presentation to ensure that the

identity of the participant is protected at all times. Therefore, while results of this project may be available for public inspection any data displayed will in no way be linked to any specific individual participating in this investigation.

Upon completion of this study, the data recorded will be securely stored for 5 years in such a way that only the researchers involved in this investigation will be able to gain access to it. Participants are most welcome to request a copy of the results of the project. Some individual results will be available immediately following testing with others available after analysis. Participants will also be able to request the results of the project as a whole and we will be available to explain and interpret specific data and how it compares to the results of the group as a whole.

### **Any questions?**

If you have any questions about our project, either now or in the future, please feel free to contact:

Kirsty Brock

Email: [K.Brock.15@unimail.winchester.ac.uk](mailto:K.Brock.15@unimail.winchester.ac.uk)

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Dr Hazel Brown

[Hazel.Brown@winchester.ac.uk](mailto:Hazel.Brown@winchester.ac.uk)

This project has been reviewed and approved by the Department of Sport Sciences Ethics Committee, University of Winchester.

## **Reproducibility of single position near infra-red spectroscopy placement in Fp1 and AF3 positions to assess prefrontal cortex activation**

### **Information Sheet for Participants**

Thank you for showing an interest in the project. Please read this information sheet carefully before deciding whether or not to participate. If you choose to participate, we thank you in advance for the time and effort you have decided to devote to our investigation. If you choose not to participate there will be no disadvantage to you of any kind and we thank you for considering taking part in this project.

#### **What is the aim of the project?**

This project is being undertaken as part of the requirements for an MPhil/PhD at the University of Winchester. The overall aim of this PhD is to investigate potential benefits of completing two tasks at the same time (dual-task) on (re) learning of upper limb movements in stroke survivors. The completion of two similar tasks at the same time has the potential to improve performance of the main task and consequently to improve learning of a skill. The specific aim of this project is to identify the optimum placement of a NIRS probe for assessment of executive function at rest and during exercise. The results of this study will inform the use of the NIRS probe for future projects.

#### **What types of participants are needed?**

We are looking to recruit males and females aged 35 or under who are in good health. Participants will need to attend for testing on three occasions, two of which will last approximately 1 hour and one of which will last approximately 2 hours.

### **What will participants be asked to do?**

Participants will be asked to attend for testing on five occasions, the length of each visit will be no more than 1.5 hours.

**Visit 1:** On the first visit participants will complete a maximal exercise test of a bike to determine exercise intensity levels for subsequent tests.

**Visit 2:** During the second visit participants will complete 4 trials of the STROOP test both at rest and during moderate intensity exercise to become familiarised with the protocol. Participants will also be familiarised with the application of the cerebral NIRS probes.

**Visits 3, 4 and 5:** The third, fourth and fifth visits will occur in a randomised order. One of these visits will involve participants attending for an EEG assessment. During this visit participants will be connected to an EEG machine and will be asked to complete 4 STROOP tests separated by a 5 minute break. On the other two visits participants will complete three resting STROOP tests separated by a 5 minute break followed by three bouts of moderate intensity cycling with a STROOP test applied during exercise. Participants will have two NIRS probes applied to different locations on the forehead throughout these two visits.

### **Can participants change their mind and withdraw from the project?**

If at any time you decide you no longer wish to participate in this project (for any reason) you may withdraw without disadvantage to yourself of any kind.

### **What data or information will be collected and what use will be made of it?**

Any data collected during this test will be used to establish profiles across the group of participants involved and these cumulative scores may be available for public inspection in research journals and/or at seminars or conferences. In addition, individual data/responses indicative of the typical response may also be presented. However, in all cases, anonymity will be strictly preserved. Participant codes will be used for all data presentation to ensure that the identity of the participant is protected at all times. Therefore, while results of this project may be available for public inspection any data displayed will in no way be linked to any specific individual participating in this investigation.

Upon completion of this study, the data recorded will be securely stored for 5 years in such a way that only the researchers involved in this investigation will be able to gain access to it. Participants are most welcome to request a copy of the results of the project. Some individual results will be available immediately following testing with others available after analysis. Participants will also be

able to request the results of the project as a whole and we will be available to explain and interpret specific data and how it compares to the results of the group as a whole.

**Any questions?**

If you have any questions about our project, either now or in the future, please feel free to contact:

Kirsty Brock

Email: [Kirsty.brock@winchester.ac.uk](mailto:Kirsty.brock@winchester.ac.uk)

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Dr James Faulkner

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Dr Hazel Brown

[Hazel.Brown@winchester.ac.uk](mailto:Hazel.Brown@winchester.ac.uk)

This project has been reviewed and approved by the Department of Sport Sciences Ethics Committee, University of Winchester.

## **Validity and Reliability of single position Near Infrared Spectroscopy probes to determine changes in prefrontal cortex activation**

### **Information Sheet for Participants**

Thank you for showing an interest in the project. Please read this information sheet carefully before deciding whether or not to participate. If you choose to participate, we thank you in advance for the time and effort you have decided to devote to our investigation. If you choose not to participate there will be no disadvantage to you of any kind and we thank you for considering taking part in this project.

#### **What is the aim of the project?**

This project is being undertaken as part of the requirements for an MPhil/PhD at the University of Winchester. The overall aim of this PhD is to investigate potential benefits of completing two tasks at the same time (dual-task) on skill performance and learning. The completion of two similar tasks at the same time has the potential to improve performance of the main task and consequently to improve learning of a skill. The specific aim of this project is to identify whether single position NIRS probes provide valid and reliable assessments of changes in cerebral oxygenation. The results of this study will inform the use of the NIRS probe for future projects.

#### **What types of participants are needed?**

We are looking to recruit males and females aged 40 or under who are in good health. Participants will need to attend for testing on six occasions, three of which will last approximately 30 minutes, one of which will last approximately 1 hour and two of which will last approximately 1.5 hours.

#### **What will participants be asked to do?**

Participants will be asked to attend for testing on six occasions, the length of each visit will be no more than 2 hours.

**Visit 1:** On the first visit participants will complete a maximal exercise test of a bike to determine exercise intensity levels for subsequent tests.

**Visits 2 and 3:** During the second and third visit participants will complete 4 trials of the STROOP test at rest and four trials of the STROOP test during moderate intensity exercise.

**Visit 4:** During the second visit participants will complete four STROOP tests at rest whilst EEG data is recorded.

**Visits 5 and 6:** During visits 3 and 4 participants will complete one resting STROOP test and one STROOP test during exercise.

### **Can participants change their mind and withdraw from the project?**

If at any time you decide you no longer wish to participate in this project (for any reason) you may withdraw without disadvantage to yourself of any kind.

### **What data or information will be collected and what use will be made of it?**

Any data collected during this test will be used to establish profiles across the group of participants involved and these cumulative scores may be available for public inspection in research journals and/or at seminars or conferences. In addition, individual data/responses indicative of the typical response may also be presented. However, in all cases, anonymity will be strictly preserved. Participant codes will be used for all data presentation to ensure that the identity of the participant is protected at all times. Therefore, while results of this project may be available for public inspection any data displayed will in no way be linked to any specific individual participating in this investigation.

Upon completion of this study, the data recorded will be securely stored for 5 years in such a way that only the researchers involved in this investigation will be able to gain access to it. Participants are most welcome to request a copy of the results of the project. Some individual results will be available immediately following testing with others available after analysis. Participants will also be able to request the results of the project as a whole and we will be available to explain and interpret specific data and how it compares to the results of the group as a whole.

### **Will I receive any compensation for my time?**

All participants will receive a £10 Amazon voucher as a thank you for their participation in this study.

### **Any questions?**

If you have any questions about our project, either now or in the future, please feel free to contact:

Kirsty Brock

Email: [Kirsty.brock@winchester.ac.uk](mailto:Kirsty.brock@winchester.ac.uk)

Telephone number: 01962 827046

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Dr Hazel Brown

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This project has been reviewed and approved by the Department of Sport Sciences Ethics Committee, University of Winchester.



**DSE**  
DEPARTMENT OF  
SPORT AND EXERCISE

## **Investigating the Impact of Dual-Tasks on Neurological Activation During Learning of a Novel Task**

### **Information Sheet for Participants**

Thank you for showing an interest in the project. Please read this information sheet carefully before deciding whether or not to participate. If you choose to participate, we thank you in advance for the time and effort you have decided to devote to our investigation. If you choose not to participate there will be no disadvantage to you of any kind and we thank you for considering taking part in this project.

#### **What is the aim of the project?**

This project is being undertaken as part of the requirements for a PhD at the University of Winchester. The overall aim of this PhD is to investigate potential benefits of completing two tasks at the same time (dual-task) on (re) learning of upper limb movements in stroke survivors. The completion of two similar tasks at the same time has the potential to improve performance of the main task and consequently to improve learning of a skill. The specific aim of this project is to identify the effects of completing two tasks (dual-task) at the same time on learning and performance of an upper limb skill task in healthy adults. This project will provide interesting information regarding the effects of dual-task performance on skill learning and performance and the mechanisms occurring in the brain when people perform two tasks whilst learning a new skill. This project will inform the design of future projects working with stroke survivors.

#### **What types of participants are needed?**

We are looking to recruit males and females between the ages of 18 and 50 years who are in good health, have no restrictions on upper limb movement or uncorrected sight/hearing problems. Participants should be willing to attend the University of Winchester for testing on six occasions which will last no more than 1.5 hours each time.

### **What will participants be asked to do?**

Participants will be asked to attend for testing on six occasions, the length of each visit will be no more than 1.5 hours. During each visit participants will complete several trials of a game on an Xbox Kinect computer system in which you move your arm and hand to complete a bowling game. Participants will be randomly assigned to a group meaning they will complete just the game, or they will complete the game whilst also completing an audio response task involving either responding to a noise by identifying when they hear it or responding to a noise by identifying whether the noise is of high or low pitch. A further dual-task condition consisting of a clock task where participants listen to a time and will need to identify whether the hands of the clock would be on the same side by indicating yes or no will also be used during baseline and retention tests.

**Visit 1:** The first visit will take approximately 1.5 hours and will be a familiarisation session allowing participants to become familiar with the laboratory surroundings and with the game that will be used for the main tests and with the NIRS equipment. Participants will initially be measured for the placement of a near infra-red spectroscopy probe (NIRS) on the forehead. Once this is positioned participants will complete three games in the control condition and one game in each of the three different dual-task conditions that will be used in the study. Participants will then complete a baseline test which will involve three games in the control, and in each of the dual-task conditions applied in a random order.

**Visits 2 and 3:** Visits 2 and 3 will be 7-10 days after visit 1 and will both take place during a 5 day period. These sessions will take approximately 1.5 hours. Participants will complete 18 game trials and will have a five minute break between each block of 3 games. All trials will be conducted wearing the NIRS probe.

**Visit 4:** The fourth visit will take place within 7 days of the second visit and will consist of six training game trials followed by a retention test consisting of game three trials in the control condition and in each of the three dual-task conditions. This session will take approximately 1.5 hours. Participants will wear the NIRS probe at all times.

**Visit 5:** The fifth visit will take place one week after the fourth visit and will take approximately 1 hour. This session will consist of three game trials in the control condition and three trials in each of the dual-task conditions. Participants will wear the NIRS probe at all times.

**Visit 6:** The sixth visit will take place four weeks after the fourth visit and will take approximately 1 hour. This session will consist of three game trials in the control condition and three trials in each of the dual-task conditions. Participants will wear the NIRS probe at all times.

### **Can participants change their mind and withdraw from the project?**

If at any time you decide you no longer wish to participate in this project (for any reason) you may withdraw without disadvantage to yourself of any kind.

### **What data or information will be collected and what use will be made of it?**

Data involving game performance and blood oxygenation measures (as determined by the NIRS) will be collected throughout each session. Any data collected during this study will be used to establish profiles across the group of participants involved and these cumulative scores may be available for public inspection in research journals and/or at seminars or conferences. In addition, individual data/responses indicative of the typical response may also be presented. However, in all cases, anonymity will be strictly preserved. Participant codes will be used for all data presentation to ensure that the identity of the participant is protected at all times. Therefore, while results of this project may be available for public inspection any data displayed will in no way be linked to any specific individual participating in this investigation.

Upon completion of this study, the data recorded will be securely stored for 5 years in such a way that only the researchers involved in this investigation will be able to gain access to it. Participants are most welcome to request a copy of the results of the project. Some individual results will be available immediately following testing with others available after analysis. Participants will also be able to request the results of the project as a whole and we will be available to explain and interpret specific data and how it compares to the results of the group as a whole.

### **Any questions?**

If you have any questions about our project, either now or in the future, please feel free to contact:

Kirsty Brock

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This project has been reviewed and approved by the Department of Sport Sciences  
Ethics Committee, University of Winchester.

## Appendix C – PAR - Q

**Participant's Consent**

I \_\_\_\_\_ consent to take in part in this research  
study titled:

The investigator has explained the full details and parameters of all tests and procedures to me, and/or I have read the Information Sheet. I confirm that I have understood what participation will involve, and confirm that I have been made aware of all the potential benefits and risks of participation.

I declare that I have completed and signed the accompanying Physical Activity Readiness Questionnaire truthfully to the best of my knowledge, and that I have never been advised to abstain from any form of exercise by a medical practitioner. I know of no reason why participation in these testing procedures might present a risk to my safety.

I understand that any medical information that I have submitted will be treated as highly confidential.

I would like to be provided with a copy of the following for my personal records  
(*please tick*):

**Information Sheet****Consent Form****PAR-Q**Signed: \_\_\_\_\_ (*Participant*)

Date: \_\_\_\_\_

Signed: \_\_\_\_\_ (Witness)

Date: \_\_\_\_\_



Date	Blood Pressure

**Physical Activity Readiness Questionnaire (PAR-Q)**

Date of Birth		Blood pressure (mmHg)	
Height (m)		Body Mass (kg)	

**Please tick either 'Yes' or 'No' for all of the following questions. If you are unsure about any question, please ask the investigator.**

			Yes	No
Are you used to vigorous exercise?				
Has your medical doctor said that you must not undertake vigorous activity?				
Do you have:	Yes	No	Yes	No
heart disease?				
frequent chest pains?				
raised blood pressure?				
episodes of excessive breathlessness?				
a persistent cough?				
asthma?				
a recent chest infection?				
			Yes	No
Do you lose your balance because of dizziness?				
Do you have episodes where you regularly lose consciousness?				
To the best of your knowledge, are you pregnant?				
Do you have any implanted electronic devices such as cardiac pace-makers or similar assistive devices?				
Do you have any other condition that may prevent you either exercising or taking part in this project? Please give details below:				

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If you have any other concerns or questions with regard to completing this form or are unsure as to your general state of health please contact the investigator in person or at the following email address:  
ed.tasker@winchester.ac.uk

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***For Official Use only***

Details of any further discussions with research subject regarding health indications stated above:

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Signed: \_\_\_\_\_ (*Participant*) Date: \_\_\_\_\_ Signed: \_\_\_\_\_ (*Lab Supervisor*)

*I declare that the information above has not changed since my first visit:*

Signed: \_\_\_\_\_ (*Participant*) Date: \_\_\_\_\_ Signed: \_\_\_\_\_ (*Lab Supervisor*)

Signed: \_\_\_\_\_ (*Participant*) Date: \_\_\_\_\_ Signed: \_\_\_\_\_ (*Lab Supervisor*)

Signed: \_\_\_\_\_ (*Participant*) Date: \_\_\_\_\_ Signed: \_\_\_\_\_ (*Lab Supervisor*)

## **Appendix D – NIRS measurement instructions**

## **NIRS Measurement Instructions**

- Take nose to cranial notch measurement
- 10% of this measurement mark Fpz
- 30% of this measurement mark Fz
- Take circumference measurement
- 5% of this measurement mark Fp2
- 15% of this measurement mark F8
- Midpoint between Fz and F8 = F4
- Midpoint between F4 and Fp2 = AF4
- Repeat for left side (Fp1 and AF3 positions)

## Appendix E – Borg scale

## Rating of Perceived Exertion

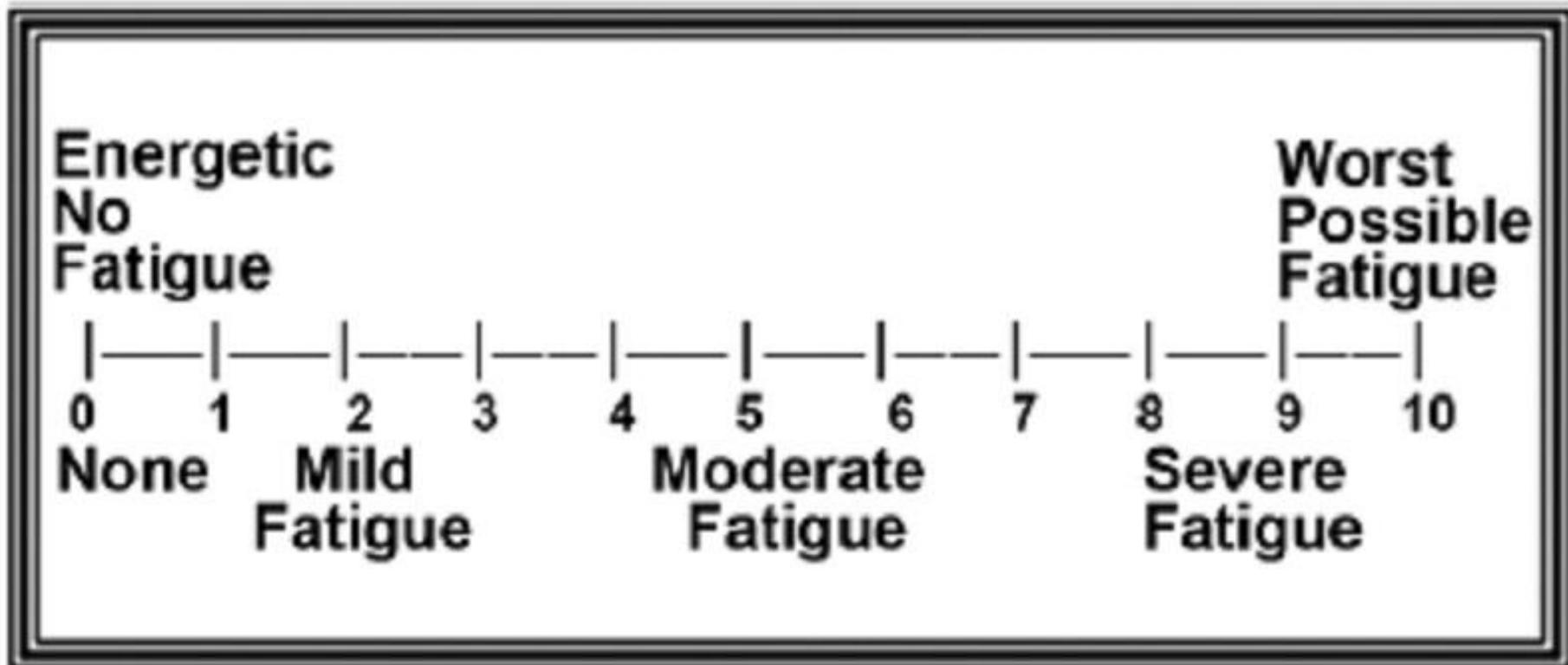
Please indicate how much exertion you were feeling during the previous block of trials.

<b>Rating</b>	<b>Perceived Exertion</b>
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

## Appendix F – Fatigue scale

## Perceived Fatigue Scale

Please indicate the level of fatigue you feel right now at the present moment by selecting a number between 1 and 10.



## **Appendix G – SPSS Output**

SPSS Output files can be viewed or downloaded from:

<https://1drv.ms/u/s!AkRu4HfssBRVkiav388mCxbEJDTn?e=Jfm5Z3>