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Effects of sex and handedness on the hemodynamic response obtained in the human prefrontal cortex

DOCTORAL DISSERTATION

Natural Sciences,
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Lyties ir rankiškumo įtaka žmogaus prieškaktinės skilties tyrimuose registruojamam hemodinaminiam atsakui

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CONTENT

LIST OF ABBREVIATIONS

AI	asymmetry index
ANOVA	one-way univariate analysis of variance
BBB	blood brain barrier
BCST	Berg Card Sorting Test
BOLD	blood oxygenation level-dependent signal
CBF	cerebral blood flow
CBV	cerebral blood volume
CNS	central nervous system
COBI	cognitive optical brain imaging studio software
CSF	cerebrospinal fluid
CT	computerised tomography
CW	continuous wave
DAQ	data acquisition module
DOT	diffuse optical tomography
DPF	differential pathlength factor
EEG	electroencephalography
ERP	event-related potentials
FD	frequency domain
FIR	finite impulse response
F-L	females left-handed
F-R	females right-handed
HbO_2	oxygenated haemoglobin
HbR	deoxygenated haemoglobin
HbT	total haemoglobin
HR	hemodynamic response
HRF	hemodynamic response function
ICV	intracranial volume
IR	infrared radiation
LI	laterality index
LP	low-pass filter
MA	motion artefact
MBLL	modified Beer-Lambert law
MC	modified comparison
MEG	magnetoencephalography
M-L	males left-handed
M-R	males right-handed

NMC	neurometabolic coupling
NVC	neurovascular coupling
NVU	neurovascular unit
OXY	total change of oxygen
PEBL	psychology experiment building language
PET	positron emission tomography
PFC	prefrontal cortex
RED	red light
RM-MANOVA	repeated-measures multivariate analysis of variance
S1	source 1
SD	standard deviation
SMAR	sliding-window motion artefact rejection algorithm
SPECT	single photon emission tomography
TCDS	transcranial Doppler sonography
TD	time domain
WCST	Wisconsin Card Sorting Test

INTRODUCTION

Modern functional neuroimaging methods cover broad spatial and temporal scales (Pouratian, Sheth, Martin, & Toga, 2003) and facilitate important exploration of the human brain's functional organisation in health and disease (Liu et al., 2015). As a result, we are witnessing enormous growth in the field of human brain research (Raichle, 2009; Toga, 2015). As a result of such growth, many field-specific research challenges have emerged. For instance, one of the significant challenges that functional neuroimaging of the human cognition faces is an incomplete understanding of typical between-subject differences (Seghier & Price, 2018).

Despite the same gross human brain structure and function, each person also has unique structural-functional features in their brain likely caused by genetic variations and individual brain plasticity; however, it is unclear to what extent this natural variability between subjects exists (Elliott et al., 2018). Part of this uniqueness is thought to be reflected in higher-order processes, namely executive functions, which simultaneously use several lower-order cognitive functions towards goal-directed action (Diamond, 2013; Poldrack & Yarkoni, 2016; Seghier & Price, 2018; Yarkoni, Poldrack, Van Essen, & Wager, 2010). At this point, sex and handedness are of great interest for decomposing the natural variability due to apparent structural and functional asymmetries concerning these biological factors in humans. There are four main research-based reasons sex and handedness matters for neuroscience: (a) the fact that gonadal hormones affect some structural and functional cerebral asymmetries (Amin, Epperson, Constable, & Canli, 2006; Hausmann, 2017; Schöning et al., 2007); (b) evident sex-related differences in specific cognitive tasks, or potentially overall different information processing strategies (Bidula, Przybylski, Pawlak, & Króliczak, 2017; Hill, Laird, & Robinson, 2014; McCarthy, Arnold, Ball, Blaustein, & De Vries, 2012; Schmitz, Lor, Klose, Güntürkün, & Ocklenburg, 2017; Zilles et al., 2016); (c) sex-related odd ratios for left-handers (Papadatou-Pastou, Martin, Munafò, & Jones, 2008); (d) the unexplained phenomenon of the collateralisation of preferred hand motor control in the left hemisphere together with language centres (for more than 90% of humans) (Hervé, Zago, Petit, Mazoyer, & Tzourio-Mazoyer, 2013; Knecht et al., 2000). It is still highly debatable how sex and handedness affect executive functions, which have been mainly attributed to the activity and structural integrity of the frontal lobes (Ayaz et al., 2012; Diamond, 2013; Miller & Halpern, 2014).

The posterior frontal cortex supports concrete rule learning, while more anterior regions along the rostro-caudal axis of the frontal cortex support learning and cognitive control at higher levels of abstraction (Badre & D'Esposito, 2009). Thus, these regions are of high interest. However, widely used neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), suffer from distortions and signal dropout *around this region* due to air inside the sinuses, particularly in the forehead where the prefrontal cortex (PFC) is located (Wise, 2013). At this point, another technique similar in terms of the origin of the obtained signal, called functional near-infrared spectroscopy (fNIRS), can be used.

fNIRS is a promising alternative to fMRI because of its portability and correspondence with other functional neuroimaging techniques across the variety of different cognitive tasks (Cui, Bray, Bryant, Glover, & Reiss, 2011; Steinbrink et al., 2006). Moreover, fNIRS is based on a phenomenon common among all functional neuroimaging techniques called neurovascular coupling (NVC). NVC is the process by which active brain regions induce a local increase in blood flow to match their energy demands via the dilation of capillaries and arterioles through various cellular signalling paths (Mishra et al., 2016). This process is expected to contribute substantially to the observed hemodynamic response (HR), which is widely presented as functional human brain maps. The unique feature of the fNIRS technique is that due to its relatively simple design, modifications to existing or the development of custom systems for specific researchers' needs are relatively simple. fNIRS was thus the preferred technique in these research projects as an ideal choice for the investigation of sex and handedness effects on the executive functioning in the PFC.

With the aim to investigate the effects of sex and handedness on cognitive functioning, there was an attempt to build a custom fNIRS device suitable for a multimodal approach in which the activity of the PFC could be measured simultaneously and specifically for the researchers' needs. Thus, the first research project presented in the dissertation covers the creation of a custom-designed continuous-wave fNIRS system for cognitive frontal lobe studies in human adults. After the building phase and before continuing with cognitive studies, it was necessary to apply a well-known, standard cognitive task to validate a device being able to capture the HR in the PFC in comparison with an existing machine. As such, the Wisconsin Card Sorting Test (WCST) was chosen, with an assumption of it not being sensitive to sex and handedness (Eling, Derckx, & Maes, 2008; Nyhus & Barceló, 2009; Overman, 2004).

Unexpectedly, a mild variation between the measured HR and participants' sex and handedness was observed. Thus, the second research project was mainly designed for investigating sex- and handedness-related differences during the WCST performance in the largest cohort to date of presumably healthy participants examined with fNIRS. No previous extensive investigations have been done on sex and handedness effects on functional neuroimaging data of WCST performance (Eling, Derckx, & Maes, 2008; Nyhus & Barceló, 2009; Overman, 2004). This is worth considering since the variation in the composition of participants' groups can be seen in the existing overview of WCST research, such as that of Nyhus and Barceló (2009). Moreover, as mentioned above, the possibility of these factors having influence cannot be neglected, although the test was previously assumed to be insensitive due to indifferent behavioural results. By this, instead of a broad investigation of sex and handedness effects on the executive functioning in the PFC with a custom build device, the dissertation is focused on the unexplored area of sex and handedness effects on the WCST-evoked HR.

Additionally, the second project also fills the gap of fNIRS-based investigation of the prefrontal lobe activity with a critical overview of possible confounding biological factors – only a few previous studies have used fNIRS for investigating the WCST (Fallgatter & Strik, 1998; Hashimoto, Uruma, & Abo, 2007; Sumitani et al., 2006), and none of these studies proposed a reasonable explanation of their conflicting findings. Moreover, it was also hypothesised that if sex and handedness have any effect on the HR evoked in PFC, these effects could be controlled by using the normalisation procedure. Moreover, normalisation in the presence of sex effects can be a simple way to capture more subtle handedness-related differences. If these presumably more dominant sex effects can be eliminated with normalisation, it would confirm that original data distribution is a combination of two overlapping distributions defined by this factor. In fact, this was the case in the second research project: during the WCST, males statistically significantly demonstrated higher concentration levels of oxygenated haemoglobin (HbO_2) in the PFC than females. Moreover, sex-related effects were mostly cancelled out by normalisation, except the identified strong relationship with handedness obtained regarding oxygenation (OXY) concentrations.

From a practical perspective, the current investigation of sex and handedness on the functional neuroimaging results of healthy participants in the WCST provide new insights not only for fundamental research but also for clinical applications. For example, If there were no sex or handedness

effects on functional neuroimaging results of the WCST performance, it could strengthen the population-level inferences from the other studies that investigate brain disorders and are known to exhibit an apparent bias for a particular sex (Amen et al., 2017; Bale & Epperson, 2017; Landry & Al-Taie, 2016), or handedness (Brandler & Paracchini, 2014; Laws, 2002; Schmitz et al., 2017). But, if there were such effects on functional neuroimaging results among healthy participants, it would be a strong reason to recommend overviewing the existing WCST studies by taking into account the composition of patient versus healthy participant groups, and could in turn potentially help at least partially explain existing discrepancies, such as unclear locations and spreading of the main functional activity regarding the WCST (Nyhus & Barceló, 2009).

In sum, the dissertation presents an in-depth study of the HR translation into scientific findings in cognitive neuroscience research, based on the frontal cortex activation, particularly during WCST performance among healthy subjects.

The aim of the study

The aim of the present study is to examine the potential effects of sex and handedness on the human frontal lobe hemodynamic response during the neuropsychological WCST in consistently-handed healthy adult participants by applying fNIRS.

The objectives of the study

The research objectives that facilitate the achievement of the dissertation aim are:

1. To assess the impact of sex and handedness on the HR of the PFC during the WCST by evaluating functional neuroimaging results;

2. To determine the effects of sex and handedness on higher cognitive functions of the PFC during the WCST by evaluating behavioural results;

3. To examine whether the prefrontal lobes in both hemispheres are activated differently during the performance of the WCST.

The dissertation statements

1. Sex and handedness have an impact on the functional neuroimaging results of WCST performance among healthy participants, although behavioural results do not show differences. This could at least partially explain discrepancies found in the other studies in the locations and spreading of the functional activity during the WCST.

2. The functional asymmetry in the prefrontal cortex during the WCST is not consistent with regard to hemispheres.

The scientific novelty of the study

The current research project examined the largest cohort to date of healthy and consistently-handed subjects performing the WCST and measured their PFC hemodynamics by applying fNIRS. Only a few previous studies have used fNIRS for investigating the WCST and demonstrated inconsistent findings, carried out in small and heterogeneous groups of healthy participants or patients (Fallgatter & Strik, 1998; Hashimoto et al., 2007; Sumitani et al., 2006). Moreover, none proposed a reasonable explanation of their contradictory findings nor performed sex-related and handedness-related evaluation.

1. LITERATURE REVIEW

1.1. Cerebrovascular regulation

The human brain represents only 2% of the total body mass. However, approximately 25% of the oxygen and 20–70% of the glucose consumed by the human body is dedicated to cerebral functions (Herculano-Houzel, 2011). The primary processes contributing to the high brain energy needs are (a) the maintenance and restoration of ion gradients, dissipated by signalling processes, such as post-synaptic and action potentials; (b) the uptake and recycling of neurotransmitters (Attwell et al., 2010). The blood supply ratio in the brain between grey and white matter is approximately 4:1 (Schmidt & Sokoloff, 2001). Explaining why the active brain cortical regions are often provided more than they require. One of the researchers poetically illustrated it as 'watering the garden for the sake of a Single thirsty flower' (Malonek & Grinvald, 1996). This overcompensation or functional hyperaemia is a fundamental phenomenon in normal brain function. It was first confirmed by Roy and Sherrington (1890) and defines the dilation of arterioles and capillaries of a brain region in response to a local episode of high neuronal activity (Fig. 1.1.1 B). Functional hyperaemia is a generalised term for the outcome of a complex cerebrovascular regulation mechanism apparent in neuroimaging.

The computational properties of the human brain arise from interactions between billions of neurons connected in multiple complex networks. However, our ability to study these networks in the healthy human brain is limited by the necessity to use non-invasive technologies. This is in contrast to animal models in which a rich, detailed view of cellular-level brain function with cell-type-specific molecular identity has become available due to recent advances in microscopic optical imaging and genetics (Figure 1.1.1 A),(Uhlirova et al., 2016). The regulation of the cerebral microcirculation is provided by the structural-functional derivative called the neurovascular unit (NVU), (Figure 1.1.1 C),(Attwell et al., 2010; Leybaert, 2005). The concept of the NVU emerged around 2001 (Iadecola, 2017). The NVU represents the interface between the vascular and neural compartments in the brain and is composed of vascular, glial and neuronal cells, such as a neurones, astrocytes, endothelial cells, and pericytes (Hawkins & Davis, 2005; M. D. Sweeney, Ayyadurai, & Zlokovic, 2016). The NVU may vary in overall structure and function depending on its location in the brain (Kowiański, Lietzau, Steliga,

Waśkow, & Moryś, 2013; Petzold & Murthy, 2011). Thus, emphasising the numerous processes involved in maintaining adequate blood flow for healthy brain activity. The NVU is essential for several cerebrovascular processes: the formation of the blood-brain barrier (BBB), the formation of neurometabolic coupling (NMC) and the most important within the scope of this dissertation – the formation of NVC (Leybaert, 2005; Leybaert, De Bock, Van Moorhem, Decrock, & De Vuyst, 2007; Petzold & Murthy, 2011). NVC is the process by which active brain regions induce a local increase in blood flow to match their energy demands via the dilation of capillaries and arterioles through various cellular signalling paths (Mishra et al., 2016). Capillary dilation generates a significant portion of the blood flow increase, evoked by neuronal activity (Hall et al., 2014), and is expected to contribute substantially to the observed HR (Lindauer et al., 2010). The HR describes the empirical observation of a physiological NVC event. In other words, an HR is a spatiotemporal picture of underlying NVC and cerebrovascular regulation at large.

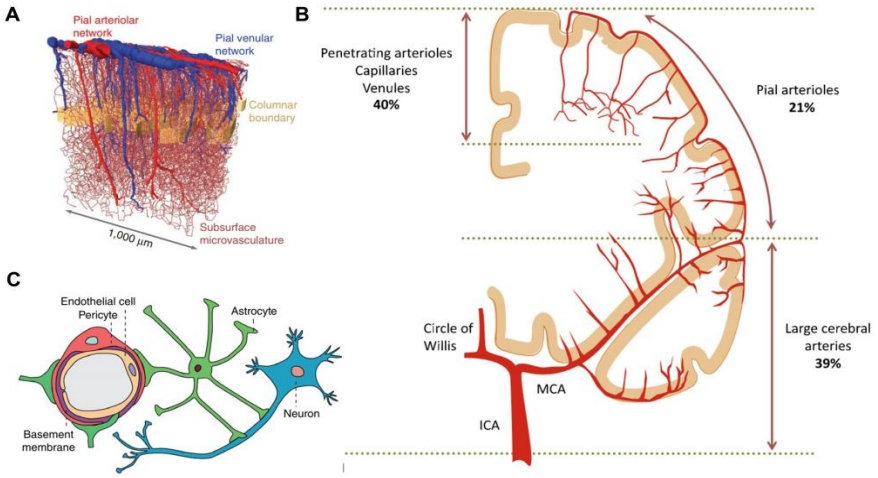

Figure 1.1.1. (**A)** A 3-D reconstruction of blood vessels within a sensory area of a mouse's brain (Blinder et al., 2013). Surface and penetrating arterioles are coloured red, venules are blue, and a golden band denotes the borders of cortical columns; (**B)** Anatomy of the cerebrovascular tree and segmental vascular resistance: The internal carotid artery (ICA) enters the skull and merges with branches of the vertebral arteries to form the circle of Willis at the base of the brain. The middle cerebral artery (MCA) takes off from the circle of Willis and supplies a vast territory of the cerebral cortex. The MCA

gives rise to pial arteries and arterioles that run on the surface of the brain, forming a densely interconnected network from which arterioles penetrating the substance of the brain originate (penetrating arterioles). Penetrating arterioles give rise to the capillary network, which feeds into the venous system returning the blood to the heart. The component (percentage) of the total vascular resistance each cerebrovascular segment offers to blood flow, which reflects their potential for flow control, is indicated. Therefore, vessels outside of the brain are responsible for 60% of the resistance and vessels within the substance of the brain for 40% (Iadecola, 2017). (**C)** Schematic representation of the NVU (Sweeney et al., 2016).

1.1.1. The origin of the HR

From a historical perspective 150 years ago, the neuroglia was thought to be only a connective material in the brain and was given an entirely passive supportive role (Kettenmann & Verkhratsky, 2008). Since then, a substantial amount of research has been published supporting the idea that the previous "neuron-centric" perspective of neuroscience is not accurate. With progress in molecular and cellular biology, it is evident that glial cells are integral to the development and maintenance of the healthy central nervous system (CNS) and play a vital role in the pathogenesis of many brain disorders (Giaume, Koulakoff, Roux, Holcman, & Rouach, 2010; Kowiański et al., 2013; Liddelow & Barres, 2017; Scemes & Spray, 2003)

Scientific focus was first given to astrocytes, as they are the most abundant population of glia in the mammalian brain (Azevedo et al., 2009; Liddelow & Barres, 2017; von Bartheld, Bahney, & Herculano-Houzel, 2016). It was proven that astrocytes are not only responsible for physical brain structuring, but also (a) are critical homeostatic cellular elements that are capable of gluconeogenesis, provide neurons with lactate, and control overall glucose levels (Bélanger, Allaman, & Magistretti, 2011; Brooks, 2009; Magistretti, 2006); (b) form a tripartite synapse with neurons and modulate synaptic activity via ion and neurotransmitter concentrations in the extracellular space (Allen & Eroglu, 2017; Haydon & Carmignoto, 2006; Krencik, van Asperen, & Ullian, 2017; Perea & Araque, 2005; Perea, Navarrete, & Araque, 2009); (c) are responsible for some immune activity, promote neuronal survival and enable re-myelination within the brain (Ayaz, Allen, Platek, & Onaral, 2008; Liddelow & Barres, 2017; Rommy von Bernha, 2016); (d) act as direct and indirect modulators of cerebrovascular tone (Ayata

& Lauritzen, 2015; Bazargani & Attwell, 2016; Filosa, Morrison, Iddings, Du, & Kim, 2016; Gratton, Chiarelli, & Fabiani, 2017; Iadecola, 2017; Mishra, 2017). Moreover, they form an equivalent to neuronal the astroglial network (Attwell et al., 2010; Giaume et al., 2010; Scemes & Spray, 2003).

Furthermore, *in vivo* animal experiments (with cats and rats) for a single-vessel hemodynamic demonstrated that pial surface arteries in cats' visual cortex (as well as neurons) show orientation responsiveness (in contrast to rats, where orientation maps are not shown in general), meaning that the propagation of vascular dilation between neighbouring columns in the brain needs to be accounted for when decoding hemodynamic signals (O'Herron et al., 2016). Another *in vivo* study of animal models (with rats and mice) show that when the sensory input increases blood flow, capillaries dilate before arterioles and are estimated to produce 84% of the blood flow increase (Hall et al., 2014). Previously, it was thought that capillaries do not significantly contribute to blood flow. Moreover, the same study identifies pericytes as significant regulators of cerebral blood flow, as they are the first vascular elements to dilate during neuronal activity, and, in turn, initiate hyperaemia. The recent studies also unexpectedly showed that vasodilators released from active neurons, interneurons and astrocytes (Hamel, 2006; Miller & Halpern, 2014) are not the only essential players in functional imaging. In fact, the role of pericytes in the CNS is as diverse as previously described with astrocytes: pericytes integrate, coordinate and process signals from their neighbouring cells to generate various functional responses that are critical for CNS functions in health and disease, including (a) regulation of the blood-brain barrier permeability; (b) angiogenesis; (c) the clearance of toxic metabolites; (d) neuroinflammation and stem cell activity; (e) initiating capillary HRs (Hall et al., 2014; Iadecola, 2017; Kisler et al., 2017; M. D. Sweeney et al., 2016).

Another non-neuron cell type crucial for inducing HRs was recently identified - vascular endothelium. Several kinds of research demonstrated that vascular endothelium could propagate upstream dilations of cerebral vessels (Andresen, Shafi, & Bryan, 2006; Chen, Bouchard, McCaslin, Burgess, & Hillman, 2011; Hannah, Dunn, Bonev, & Nelson, 2011; Iadecola, 2017; Rosenblum, 1986). The *in vivo* study by Chen and colleagues, 2014 (Chen, Kozberg, Bouchard, Shaik, & Hillman, 2014) demonstrated that spatially selective endothelial disruption with light-dye treatment in rats' somatosensory cortex significantly attenuated the HR by blocking the retrograde dilation. The early stage and the peak of hyperaemia were affected

the most, meaning that neurons, astrocytes, pericytes and endothelial cells are all involved in forming HR detected by functional neuroimaging.

There are many other scientific sources regarding cellular, molecular biology and NVC that could be discussed. For example, new pieces of evidence suggest that another critical component of the NVU is interneurons that transduce signals from perivascular nerves. The crucial role of perivascular nerves is to regulate the cerebrovascular tone, influencing overall brain perfusion. Neurovascular coupling is then determined by the chemical signals released from activated perivascular nerves and astrocytes and together alter a vascular tone to adjust local perfusion in accordance to the brain activity (Hamel, 2006; Walsh, 2016). However, even with the given three *in vivo* examples, it is evident that the main drivers of NVC, and temporal properties of the HR associated with it, strongly depend on the spatial location along the cerebral vasculature (Iadecola, 2017). These new findings allow the re-evaluation of how spatiotemporal specificity might be improved alongside the technological progress of functional neuroimaging.

1.1.2. Signal transduction path in the NVU

In previous decades, the idea that elevations of calcium concentrations in astrocytes may release transmitters that regulate neuronal and vascular functions was controversial. This changed when numerous contradictions were reported and had been resolved between different studies (Bazargani & Attwell, 2016). Including the dispute of glia to neuron ratio being 1:1 (Azevedo et al., 2009; von Bartheld et al., 2016) from the previously famous claim of 10:1. Moreover, shortly after the discovery that glutamate triggers an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$), it was suggested that there might be a mechanism by which calcium signalling propagates towards astrocyte endfeet and stimulates the release of vasoactive messengers (Attwell et al., 2010). Vasoactive messengers can cause vasodilatation (most of the neurotransmitters, nitric oxide, prostaglandins, epoxyeicosatrienoic acids, lactate, adenosine etc.) or vasoconstriction (norepinephrine, 20-HETE etc.) of arterioles (Giaume et al., 2010; Kowiański et al., 2013; Leybaert, 2005; Petzold & Murthy, 2011; Scemes & Spray, 2003). The current understanding suggests that astrocytes, as well as neurones, should be divided into three spatial compartments, such as processes, soma and endfeet (Bazargani & Attwell, 2016). In this way, the release of specific vasoactive messengers in the endfeet is explained by an overall summation of ($[Ca^{2+}]_i$) in the soma and

processes. The response may differ regarding the frequency, kinetics, spatial spreads and interaction with other cellular messengers (Bazargani & Attwell, 2016). As previously discussed, astrocytes are not the only cells that are involved in HRs, but they continue to be considered the main drivers of NVC.

In sum, the conceptual biophysical scheme of the biological signal transduction path in the NVU (Figure 1.1.2) is presented. Figure 1.1.2 summarises how metabolic and physiological events (NMC and NVC) via a calcium concentration elevation cause the HR. This, in turn, can be observed with functional neuroimaging techniques as evidence of underlying neural activity. The conceptual biophysical scheme inevitably assumes neural activity-derived NVC. Nonetheless, recent findings of astrocytes have added another level of complexity to our understanding of brain function: astroglial metabolic networks may sustain or suppress neuronal activity (Giaume et al., 2010). For simplicity, the scheme does not account for it or discuss it thoroughly, as more studies have to be done to generalise new findings (Giaume et al., 2010; Walsh, 2016). However, the idea should be kept in mind for the critical evaluation of current functional neuroimaging methods, and the proposed conceptual biophysical scheme (Figure 1.1.2) should be used as a tool for a brief exploration. In contrast, Figure 1.1.3 demonstrates the detailed characterisation of different types of calcium transients in different parts of the astrocyte.

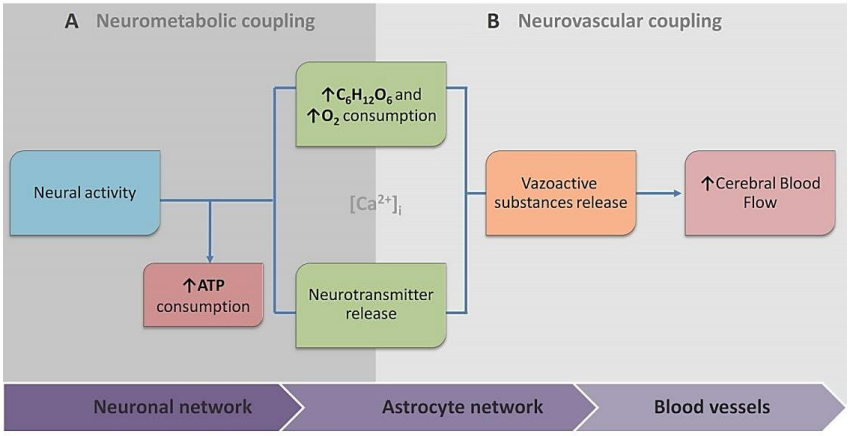

Figure 1.1.2. The conceptual biophysical scheme of the biological signal transduction path in the NVU. A) NMC; B) NVC. Both neurones (neurotransmitter release) and astrocytes (glucose and oxygen consumption) respond to increased extracellular glutamate, and intracellular calcium to

transmit direct and indirect vasoactive signals for the appropriate blood delivery and distribution in the electrically active brain area (Cinciute, 2019).

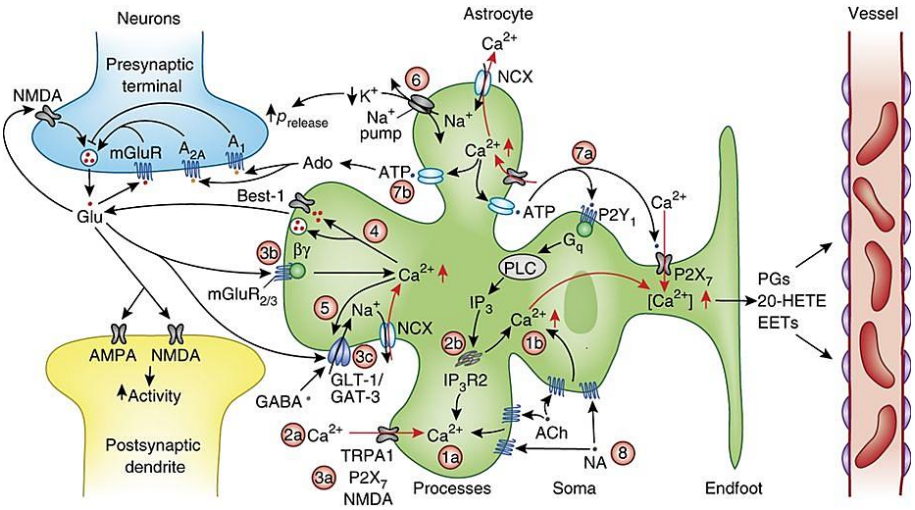

Figure 1.1.3. $[Ca^{2+}]_i$ transients in the processes of astrocytes (1a) differ from those in the soma (1b) regarding the frequency, kinetics and spatial spread. 2. $[Ca^{2+}]_i$ transients in the processes of astrocytes depend roughly equally on Ca^{2+} entry (2a) from the extracellular space through ion channels (40%) and on Ca^{2+} release from intracellular stores (60%), while those in the soma (2b) depend largely (90%) on Ca^{2+} release from the intracellular stores. 3. $[Ca^{2+}]_i$ transients can be generated by Ca^{2+} entry through spontaneously opening TRPA1 channels or neurotransmitter-gated channels (3a), by mGluR2 or mGluR3 (3b), and by neurotransmitter uptake raising $[Na^+]_i$ and reversing Na^+/Ca^{2+} exchange (3c). 4. $[Ca^{2+}]_i$ rises may release transmitters via ion channels, such as Best-1, as well as via exocytosis. 5. $[Ca^{2+}]_i$ rises to alter the surface expression of neurotransmitter transporters. 6. Activation of Na^+/Ca^{2+} exchange by a $[Ca^{2+}]_i$ increase can raise $[Na^+]_i$ and activate the sodium pump, lowering $[K^+]o$ and nearby hyperpolarising neurons. This increases the release probability (prelease) for action potential–driven vesicle release and thus decreases synaptic failure rate. 7. ATP released by a $[Ca^{2+}]_i$ increase may act on P2X or P2Y receptors to raise $[Ca^{2+}]_i$ farther along the cell, propagating a Ca^{2+} wave along the cell (7a), or be converted to adenosine, which acts on presynaptic receptors to increase (A_{2A}) or decrease (A_1) transmitter release (7b). 8. Noradrenaline (NA) released from locus coeruleus neurons, and acetylcholine (ACh) released from nucleus basalis neurons produce large $[Ca^{2+}]_i$ increases in astrocytes, (Bazargani & Attwell, 2016)

1.2. Functional neuroimaging using light

The field of neuroimaging has undergone phenomenal growth in recent years within the area of brain imaging. Optical imaging is one of the techniques that have widely extended its applications due to the ability to monitor brain activation by robustly measuring cortical brain activation (Figure 1.2.1.). Using light to study the brain noninvasively is made possible by the fact that many biological tissues, including human skin and bone, are relatively transparent to near-infrared light (Pouratian et al., 2003). The exploitation of this "near-infrared window", starting with the pioneering work of Jöbsis in 1977 (Jöbsis, 1977), resulted in various types of optical imaging. This is mainly due to the portable and non-invasive nature of the technique that allows many of the challenges associated with performing neuroimaging studies in humans to be overcome. Although there is a range of terminology, the approach is most commonly referred to as the near-infrared measuring technique type it is based on. There are three main types that dominate: (a) continuous wave (CW) – based on constant tissue illumination, directly measures light attenuation through the head; (b) frequency-domain (FD) – illuminating the head with intensity-modulated light, measures both attenuation and phase delay of emerging light; (c) time-domain (TD) – detects the shape of the short pulse after propagation through tissues (Mansouri & Kashou, 2012). However, the continuous wave type-based devices are most common due to their simple implementation. Within the scope of the thesis, the following chapter will refer specifically to continuous-wave functional near-infrared spectroscopy (CW-fNIRS, or simply fNIRS), although the most of the presented methodological background may be generalised for other optical imaging techniques as well.

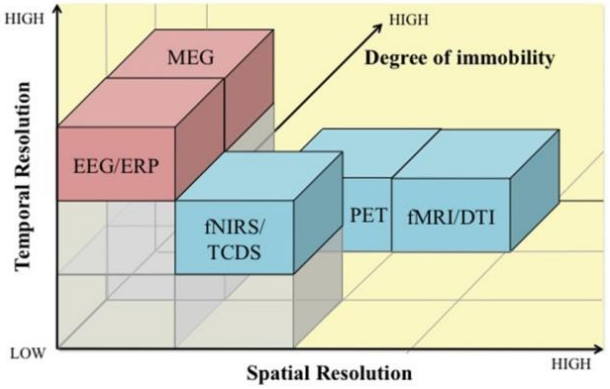

Figure 1.2.1. Comparison of electromagnetic (pink) and hemodynamic (blue) neuroimaging techniques based on temporal resolution (x-axis), spatial resolution (y-axis), and degree of immobility (z-axis). EEG, electroencephalography; ERPs, event-related potentials; MEG, magnetoencephalography; fNIRS, functional near-infrared spectroscopy; TCDS, transcranial Doppler sonography; fMRI, functional magnetic resonance imaging; DTI, diffusion tensor imaging; PET, positron emission tomography (Mehta & Parasuraman, 2013).

1.2.1. Fundamentals of optical tissue properties

Before understanding the principles of optical imaging, it is essential to acknowledge the various physical phenomena that are affecting light propagation through layers of the human brain. The interaction between light and biological tissue is characterised mainly by the optical properties, such as the phenomena of absorption, scattering and reflection (Cope & Delpy, 1988). Absorption depends on the molecular structure of chromophores and is characterised by an absorption coefficient μ_a. Scattering describes the deviation of photons in the tissue from the former trajectory. Several variables may characterise the scattering effect, as it depends on many factors, such as the molecular size, selected light wavelength and refractive index regarding the composition of tissue layers in a sample. Fortunately, in optical imaging the scattering coefficient μ_s, often may be defined by simplifying it to the exponential decay rate of light intensity with the distance travelled in the sample due to scattering effects. Both absorption and scattering play a critical role in light attenuation in biological tissues, while reflection is more involved in the back-scattering of photons and creation of banana-shaped light beams

(Dehghani & Delpy, 2000; Head & Pierson, 2009; Mansouri, L'huillier, Kashou, & Humeau, 2010; Okada et al., 1997).

Each light-absorbing compound not only has a unique absorption spectrum, but its concentration can vary with time, reflecting physiological changes in the tissue. Thus, since tissues are heterogeneous in composition, one needs to know the optical tissue characteristics for the various tissue constituents or an averaged value for the tissue as a whole to make accurate measures. Those compounds are called chromophores. Within the near-infrared range of light, the biological tissues are transparent enough that the optical imaging would allow the detection of the two main chromophores, HbO_2, and deoxygenated haemoglobin (HbR). Chromophores have a specific absorption coefficient, which is experimentally measured and describes the level of absorption at each wavelength (Cope, 1991; J. G. Kim & Xia, 2005). The absorption coefficients also define the selection of optimal wavelengths implemented in the fNIRS instrumentation.

The choice of wavelengths for fNIRS studies is complicated and directly impacts the quality of the acquired data. The "optical window" used for optical imaging is bounded roughly between 650 nm and 930 nm (Figure 1.2.2). Literature sources may refer to the different width of this window as follows: at lower wavelengths, absorption by the haemoglobin limits the depth of penetration in a tissue, and at higher wavelengths, absorption is dominated by water. The instrumentation of fNIRS typically employs wavelengths on either side of the isobestic point of haemoglobin at 800 nm, where the specific extinction coefficients of HbO_2 and HbR are equal. It is known that certain wavelength combinations can result in 'crosstalk' between the chromophores. Thus, the favourable wavelengths were determined. Several studies (Boas, Dale, & Franceschini, 2004; Strangman, Franceschini, & Boas, 2003; Yamashita, Maki, & Koizumi, 2001; Uludağ, Steinbrink, Villringer, & Obrig, 2004) have shown that a pair of wavelengths at 650–720 nm and 730–930 nm provides a superior separation between HbO_2 and HbR. In addition, the physiological noise levels at these ranges are the smallest and give the best signal-to-noise ratio (Uludağ et al., 2004). A more recent review article by (Scholkmann et al., 2014) and the recent study by (Arifler, Zhu, Madaan, & Tachtsidis, 2015) offer a more detailed discussion of this issue.

The light migrates from sources to detectors located on the head, by travelling through the skin, skull and underlying brain tissue. To quantify the chromophore concentrations, it is necessary to determine the light path length. Unfortunately, the optical tissue properties make the mean optical path length

significantly longer than the physical distance between the light source and the detector. As the light source-detector distance is geometrically fixed, and other factors can be theoretically modelled or measured empirically, thus providing the approximate value of the light path, or differential pathlength factor (DPF(λ)),(Dehghani & Delpy, 2000; Duncan et al., 1995; Head & Pierson, 2009; Kohl et al., 1998; Mansouri et al., 2010). Other important factors to quantify the concentrations of chromophores are the use of at least two wavelengths, as both compounds are measured simultaneously (Cope & Delpy, 1988; Kocsis, Herman, & Eke, 2006).

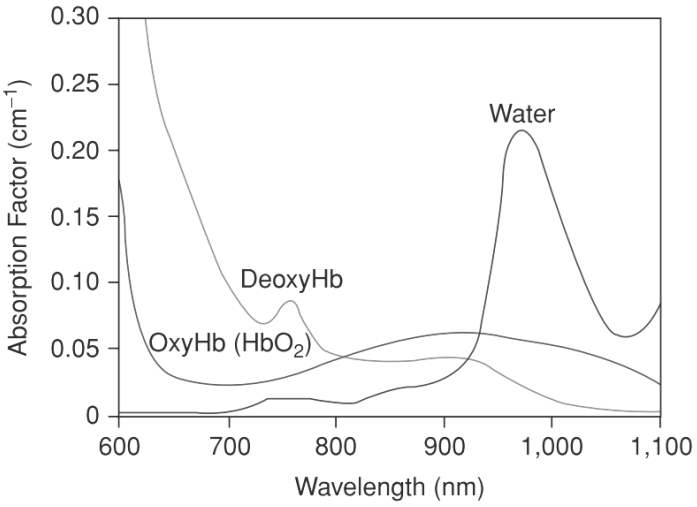

Figure 1.2.2. Absorption window of near-infrared light in biological tissues. The absorption spectrum of oxy-Hb, deoxy-Hb and water according to the light wavelength (Izzetoglu, Bunce, Izzetoglu, Onaral, & Pourrezaei, 2007).

The largest source of light loss in this path is due to the skin pigmentation and hairiness (Branco, 2007). Both thin structures strongly affect data acquisition, as the pigment melanin highly absorbs the transmitted light (Branco, 2007; Kovalenko, Roskosky, Freedman, & Shuler, 2015). In contrast, even though the adult skull is quite thick compared with other layers, its effect on the light attenuation is relatively small, and the presence of a small quantity of blood in a bone has little impact on the overall light absorption.

Further, beneath the skull, there are three layers of connective tissue membranes that envelope entirely and protect the CNS. The scattering properties of the meninges depend on its structure, as the *dura matter* is a two-

layered tough and fibrous tissue, forming a sack around the brain; the *arachnoid mater* is a delicate fibrous membrane; and the *pia mater* is delicate and highly vascularised. In summary, the overall effect of these membranes is low absorbing (Branco, 2007). However, in contrast, the cerebrospinal fluid (CSF) is optically very similar to water and thus even a thin layer potentially affects the light beam profile and the optical scattering in the brain (Branco, 2007; Dehghani & Delpy, 2000; Mansouri et al., 2010).

The light is also scattered differently through the several centimetres of *in vivo* brain tissue, especially between white and grey matter. In general, the grey matter is almost exclusively neuron bodies, whereas the white matter is almost exclusively axons. White brain matter contains a great deal of myelin (lipid-rich structures); therefore, the light scattering in white matter is approximately four times higher than that in grey matter (Branco, 2007; Cope, 1991) For this reason, the optical method is not considered very suitable for exploring the sub-cortical structures (Boas, Elwell, Ferrari, & Taga, 2014; Branco, 2007; Cope, 1991). The results of fNIRS spatial sensitivity profiles (Mansouri et al., 2010) support this claim. These kinds of studies rarely cover non-neural cell populations in the brain in general, supporting the idea that tissue properties as a whole are more important than individual aspects.

Knowledge of optical properties of the human brain tissues is essential to model the fNIRS signal accurately. Thus, from the perspective of clinical applications, tissue properties should be thoroughly considered before applying fNIRS for specific clinical cases. For example, the folding geometry of the cerebral cortex (Chuang, Chen, Hsieh, Liu, & Sun, 2013), as well as degeneration, may substantially affect the light propagation and thus impact results from subjects with certain brain diseases. Moreover, these factors need to be considered to engineer functional fNIRS devices.

1.2.2. The modified Beer-Lambert law (MBLL)

Most optical researchers are interested in absolute changes in the amplitude of chromophore concentration, while others have quantified it and expressed in concentration changes (μM), or haemoglobin signals (mM*mm). In any case, in fNIRS, the translation of variations in light attenuation into concrete measures relies on the application of a Modified Beer-Lambert law (MBLL) (Delpy et al., 1988). The original equation of the Beer-Lambert law has to be corrected, as the loss of light through scattering and the increased optical path length needed to be incorporated into the existing attenuation equation (Delpy

et al., 1988). In other words, the MBLL assumes homogeneous light absorption and constant optical scattering effects, even it is not quite accurate (Baker et al., 2014; Kocsis et al., 2006; Sassaroli & Fantini, 2004):

$$\Delta A = \alpha \cdot \Delta c \cdot L \cdot DPF, \quad (1)$$

where A is light attenuation, α is the specific absorption coefficient a given tissue ([molar^{-1}, mm^{-1}]) and c is the concentration of the chromophore of interest. L is the distance of source-detector separation, and DPF is the differential pathlength factor. When two wavelengths are used (λ_1 and λ_2), simultaneously the equations can be constructed as:

$$\Delta A^{\lambda_1} = L_{\lambda_1} \cdot DPF \left(\alpha^{\lambda_1}_{[HbO_2]} \Delta[HbO_2] + \alpha^{\lambda_1}_{[HbR]} \Delta[HbR] \right), \quad (2)$$

$$\Delta A^{\lambda_2} = L_{\lambda_2} \cdot DPF \left(\alpha^{\lambda_2}_{[HbO_2]} \Delta[HbO_2] + \alpha^{\lambda_2}_{[HbR]} \Delta[HbR] \right), \quad (3)$$

By this, the changes in HbO_2 and HbR concentrations can be resolved as follows, relating that the change in light attenuation is proportional to the changes in the concentrations of chromophores:

$$\Delta[HbO_2] = \frac{\alpha^{\lambda_1}_{[HbR]} \left(A_{\lambda_2}/L_{\lambda_2}\right) - \alpha^{\lambda_2}_{[HbR]} \left(A_{\lambda_1}/L_{\lambda_1}\right)}{\alpha^{\lambda_1}_{[HbR]} \alpha^{\lambda_2}_{[HbO_2]} - \alpha^{\lambda_2}_{[HbR]} \alpha^{\lambda_1}_{[HbO_2]}}, \quad (4)$$

$$\Delta[HbR] = \frac{\alpha^{\lambda_1}_{[HbO_2]} \left(A_{\lambda_2}/L_{\lambda_2}\right) - \alpha^{\lambda_2}_{[HbO_2]} \left(A_{\lambda_1}/L_{\lambda_1}\right)}{\alpha^{\lambda_1}_{[HbO_2]} \alpha^{\lambda_2}_{[HbR]} - \alpha^{\lambda_2}_{[HbO_2]} \alpha^{\lambda_1}_{[HbR]}}, \quad (5)$$

Subsequently, the total change of oxygen (OXY) is defined as

$$\Delta[O_2] = \Delta[HbO_2] - \Delta[HbR], \quad (6)$$

and total haemoglobin (HbT) is defined as

$$\Delta[Hb_{total}] = \Delta[HbO_2] + \Delta[HbR]. \quad (7)$$

1.2.3. Methodological issues and comparison with fMRI

Despite all the advantages of functional neuroimaging with fNIRS, there are technique-related concerns associated with it as well, such as optical signal contamination from extra-cerebral tissues, sensitivity to motion artefacts (MA) and a lack of spatial normalisation (Caldwell et al., 2016; Singh, Okamoto, Dan, Jurcak, & Dan, 2005; Tachtsidis & Scholkmann, 2016).

Both HbO_2 and HbR can be influenced by systemic physiological changes (occurring in the cerebral and/or the extra-cerebral compartment). The degree of influence often varies and also depends on the origin and type of systemic change (Tachtsidis & Scholkmann, 2016). The physiological noise is typically much slower than the sample rate of an fNIRS system and is

associated with arterial pulse, respiration and heart rate fluctuations (Boas et al., 2014). These artefacts may be removed with simple band-pass filters. Other like extra-cranial hemodynamic (Kirilina et al., 2012; Tachtsidis & Scholkmann, 2016) require more sophisticated methods like signal regression with additional data from short distance channels or the mean signal removal.

MA cause distortion in fNIRS, like in any other non-invasive neuroimaging technique. There are two types of MA: short spike-like and baseline shifts artefacts. Short spike-like artefacts can be detected by high amplitudes, while baseline shifts are particularly troubling due to their low frequency, and most motion-correction methods imply only a general regression of the slope. Most MA occur when the fNIRS sensor, light sources and/or detectors slide from their original attached location or lose contact with the skin. The pressure applied to the sensor pad or the light sources and detectors may also vary with time due to head movements, thus partially causing a baseline shift (these drifts are also partly caused by thermal effects in the instrumentations). Various MA detection and removal algorithms are proposed to overcome these issues (Ayaz, Izzetoglu, Shewokis, & Onaral, 2010; Izzetoglu, Chitrapu, Bunce, & Onaral, 2010; Izzetoglu, Devaraj, Bunce, & Onaral, 2005; Tak & Ye, 2014).

Another major concern is that common spatial normalisation in fNIRS as it exists in fMRI is not available, meaning that the surface location of fNIRS optodes lack anatomical specificity to the brain regions-of-interest. A few recent studies have suggested approaches to overcome this challenge. Augusto and colleagues (Augusto, Morais, & Balardin, 2018) suggest using predefined positions on 10–10 and 10–5 systems (Jurcak, Tsuzuki, & Dan, 2007) according to a set of brain regions of interest, and based on the two head atlases guided by functional magnetic resonance imaging. Another approach by Xiao and colleagues (Jiang et al., 2018) also suggests converting transcranial optode locations, but in this case traditional brain atlases are projected onto the skull (Jiang et al., 2018). Despite important advancements, both approaches are not yet applicable for all commercially available devices, and do not solve the issue of a lack of spatial normalisation due to different brain morphology.

Comparison of fNIRS with fMRI

fNIRS and fMRI are based on a common underlying phenomenon termed NVC, but have different capacities to explore human brain functions (Hillman, 2014; Huneau, Benali, & Chabriat, 2015; Phillips, Chan, Zheng, Krassioukov,

& Ainslie, 2016). The fMRI is more common in general because of its broad application possibilities and historical background, especially in clinical practice (Glover, 2011). The fNIRS was primarily used for the bedside monitoring of infants, and other fields where fMRI was not applicable. Thus, only quite recently fNIRS became an equivalent method for investigating human cognitive brain functions (Boas et al., 2014), and there has been an increasing number of combined functional fMRI-fNIRS studies. According to the PubMed database search for the terms fMRI and fNIRS in the title/abstract field and restricted to the results of the articles and review papers published between 1977/01/01 and 2018/06/06, a total of 752 documents with human participants were identified. After additional restriction to search for these terms only in the title field, a total of 11 documents remained (Anwar et al., 2013; Cui et al., 2011; Frederick, Nickerson, & Tong, 2012; Gagnon et al., 2012; Maggioni et al., 2013; Okamoto et al., 2004; Sasai et al., 2012; Sato et al., 2013; Strangman, Culver, Thompson, & Boas, 2002; Tong & Frederick, 2010; Toyoda et al., 2008). A few were straightforward comparisons, which have been conducted for a variety of cognitive tasks to illustrate similarities and differences between fNIRS and fMRI capabilities (Cui et al., 2011; Steinbrink et al., 2006). This is crucial for advancing cognitive and behavioural research because despite the common underlying phenomenon both methods have their own strengths and limitations associated with a biophysical and physiological signal origin (S.-G. Kim & Ogawa, 2012; Scarapicchia, Brown, Mayo, & Gawryluk, 2017).

The comparison of how the same physiological process of NVC originates as a HR measured using fNIRS (A) and the hemodynamic response function measured using fMRI (B) can be found in Figure 1.2.3. The example of HR (A) is based on measuring the composition of the total cerebral blood volume in the particular brain area. Blood Oxygen Level-Dependent (BOLD) imaging with fMRI is based on the paramagnetic deoxyhaemoglobin decrease in *T2** contrast relative to the situation with diamagnetic oxyhaemoglobin),(Bandettini & Wong, 1995; Chavhan, Babyn, Thomas, Shroff, & Haacke, 2009). With fNIRS both oxygenated haemoglobin $\Delta[HbO_2]$ and deoxygenated haemoglobin Δ[HbR] concentration changes are observed simultaneously. In addition, the Δ[HbR] curve in Figure 1.2.3 A, could be seen as a BOLD signal representation in Figure 1.2.3 B, even though it is not straightforward due to other physiological contributors captured in the BOLD response (such as regional cerebral blood flow and volume) (Arthurs & Boniface, 2002; S.-G. Kim & Ogawa, 2012).

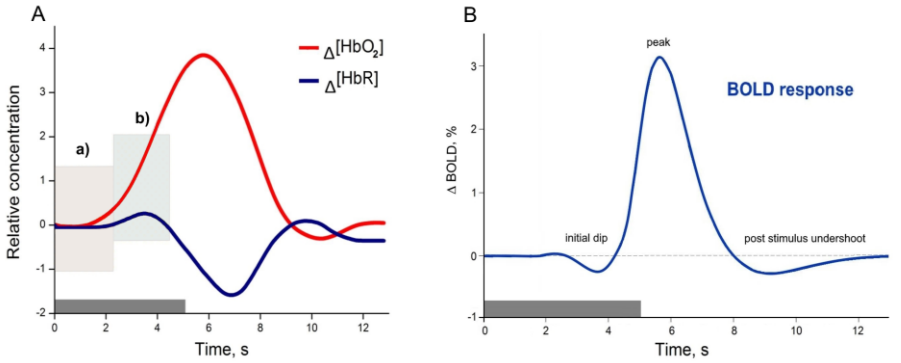

Figure 1.2.3. Examples of a canonical HR **(A)** and HR function (HRF),(**B**). Neural activity from 0 to 5 sec (grey bar) causes neurometabolic and later NVC, which can be seen as a delay of response (around 2 s). Box in HR **(A)** indicate a) small inflow of $\Delta[HbO_2]$, when the total blood volume is still relatively unchanged (caused due to increased cerebral blood flow), and later b) $\Delta[HbO_2]$ increases rapidly due to functional hyperaemia caused by vasodilatation. The small increase of Δ[HbR] occurs due to insufficient washout when the cellular oxygen demand exceeds the current supply in a tissue. The canonical example of a HR is based on measuring the composition of cerebral blood volume via chromophore concentration changes (oxy-Hb and deoxy-Hb). fNIRS studies can directly measure both oxy-Hb and deoxy-Hb) (Venclove, Daktariunas, & Ruksenas, 2015). In contrast, the canonical HRF from the BOLD method represents magnetic field change in response to the Δ[HbR] curve (B) and is relative to the baseline (Cinciute, 2019).

Because of the different aspects of HR that are captured, and sources of noise that are involved, regional hemodynamic changes measured by both methods are modelled differently. Regarding fNIRS, the extended version of an existing computational model of cerebral physiology, "BrainSignals" should be considered the most prominent (Caldwell et al., 2016). It incorporates components of (a) hemodynamic ; (b) mitochondrial brain metabolism; (c) brain oxygen consumption; (d) scalp hemodynamic . This model also joins haemoglobin-based and the cytochrome c oxidase redox state-based measurements (which are out of scope in the dissertation, but are a promising branch of optical brain measurements (Tachtsidis & Scholkmann, 2016). Meanwhile, most models of the BOLD response are based solely on cerebral blood flow, cerebral blood volume and the local metabolic rate of

oxygen consumption (S.-G. Kim & Ogawa, 2012; Arthur W. Toga, 2015). These models depict the transient hemodynamic and oxygenation changes in the activated cerebral area; they also mimic some of the physiological mechanisms of functional hyperaemia and are extensively discussed by Huneau et al. (2015). The authors noted that despite the accumulation of new findings, NVC has surprisingly been forsaken in modelling functional neuroimaging, especially in humans. Nonetheless, significant results from cellular biology and *in vivo* brain physiology are still rather offered to be considered than provided for implementing in existing computational modelling. Considering this, it is plausible that some inconsistencies between neuroimaging studies, as well as the contradiction between behavioural and neuroimaging results in some investigations, may be due to neural and vascular response mismatches, rather than because of underlying different cognition processes. Several recent scientific findings in animal (Lindauer et al., 2010) and explanatory molecular human biology models (M. D. Sweeney et al., 2016) support this. However, these studies investigated the pathophysiological changes of NVC, while the underlying mechanisms of normal NC are still not fully understood.

1.3. Investigation of the human cognitive functions

Although the hemispheres are similar in appearance, they are not entirely symmetrical in structure nor equivalent in function (Hugdahl & Westerhausen, 2010; Kandel, Schwartz, & Jessell, 2000). Each hemisphere of the human brain is concerned primarily with sensory and motor processes on the contralateral (opposite) side of the body. More interestingly, split-brain research has shown that each disconnected cerebral hemisphere could process information simultaneously and independently (Zaidel, Clarke, & Suyenobu, 1990). Meanwhile, the normal human brain's functioning and cognition is based on highly integrative abilities, such as attention, orientation, memory, gnosis, executive functions, language and social cognition. At this point, a significant part of scientific research on cognition and mental theories is devoted to discuss sex and handedness effects, often related to functional lateralization and behavioural traits (Friedman & Miyake, 2017; Yuan & Raz, 2014; Zilles et al., 2016). One reason it has attracted considerable attention is because there are plenty of neurological and mental disorders showing a clear predisposition for sex (Amen et al., 2017; Bale & Epperson, 2017) or handedness (Brandler & Paracchini, 2014; Schmitz et al., 2017). Often, they

are somewhat supported by structural and genetic evidences. But despite extensive scientific research, even normal biological mechanisms leading to between-subject differences regarding sex and handedness remain somewhat unclear.

1.3.1. Sex, handedness and the human brain structure

There are many studies about the relationships between common objective factors, such as sex and handedness and the human brain structure and function, and thus many conflicting findings (Good et al., 2001; Arthur W. Toga & Thompson, 2003).

Among the most prominent observations of gross structural asymmetries of the human brain are the right frontal and left occipital protrusions (Figure 1.3.1. A). Because of this prenatally established pattern, the left occipital lobe is extended across the midline (over the right occipital lobe), bending the interhemispheric fissure toward the right. Consequently, frontal structures on the right are also torqued forward relatively to their counterparts on the left and cause apparent structural asymmetries, or shift on the frontal–occipital axis (A. W. Toga, Narr, Thompson, & Luders, 2009; Arthur W. Toga & Thompson, 2003),(Figure 1.3.1). This structural feature is a simplistic example of why sophisticated tools and brain atlases were development to register the individual brain into a common reference space before evaluating its functioning: a particular location greatly various from brain to brain in a same space, not to mention the related morphological brain differences, such as brain volume, surface and cortical thickness (Hugdahl & Westerhausen, 2010; A. W. Toga et al., 2009; Arthur W. Toga, 2015; Arthur W. Toga & Thompson, 2003). These are important features, especially when considering biological characteristics, such as age, sex, environmental factors, healthy and disease modulated brain asymmetry and combinations thereof (Hugdahl & Westerhausen, 2010; Arthur W. Toga, 2015). For instance, the most recent and yet the largest-ever analysis of the human cerebral cortical asymmetry and its variability across individuals were assessed in MRI scans of 17,141 healthy individuals from 99 datasets worldwide (Kong et al., 2018). The results from this study not only revealed widespread asymmetries both at hemispheric and regional levels, but also in the regions involved in lateralised functions, including language and visuospatial processing – often associated with morphological and cognitive sex differences (J. S. Allen, Damasio, &

Grabowski, 2002; J M Goldstein et al., 2001; Lenroot & Giedd, 2010; Sacher, Neumann, Okon-Singer, Gotowiec, & Villringer, 2013).

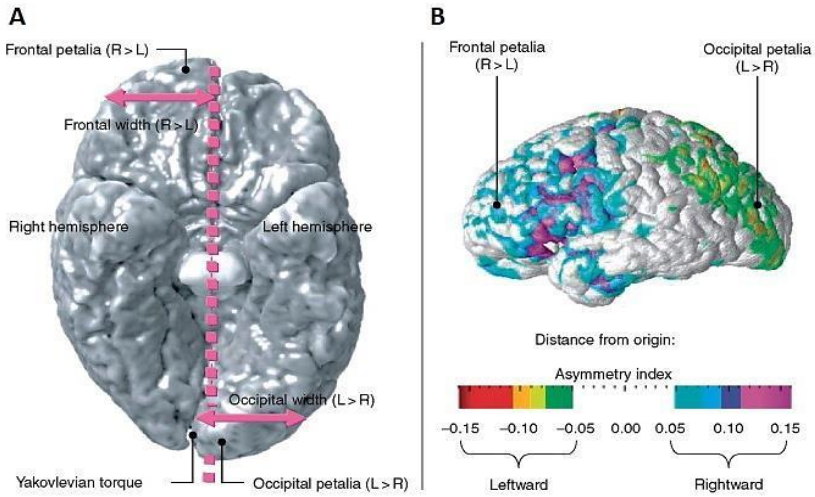

Figure 1.3.1. Petalia asymmetry. (**A**) A 3D rendering of the inferior surface of the human brain exaggerated to illustrate prominent asymmetries found in the gross anatomy of the two brain hemispheres. Noticeable protrusions of the hemispheres, anteriorly (R > L) and posteriorly (L > R), are observed, as well as differences in the widths of the frontal (R > L) and occipital lobes (L > R). A twisting effect is also observed, known as Yakovlevian torque, in which the left occipital lobe is splayed across the midline and skews the interhemispheric fissure in a rightward direction. (**B**) The magnitude and direction of hemispheric shape differences, which are estimated by measuring distances from a central point (origin) in the brain to thousands of spatially equivalent cortical surface locations in each hemisphere and by comparing these distances using an asymmetry index. The colour scale illustrates anterior protrusions of hemispheric shape in the right hemisphere and posterior protrusions of hemispheric shape in the left hemisphere in one individual. It is modified from Toga et al. (2009).

The variability in brain asymmetry was related to sex, age and intracranial volume (ICV), but not to handedness (Figure 1.3.2). The lack of handedness effects on the human brain structure is consistent with the previous meta-analysis of 106 left-handed subjects and 1960 right-handed subjects (Guadalupe et al., 2014). And although specific gene products were

proven to mediate the development of brain and body asymmetry, the real genetic role thereof in hand preference remains ambiguous and unconfirmed with structural brain meta-analysis studies (Brandler & Paracchini, 2014; Corballis, 2014; Robinson, Hurd, Read, & Crespi, 2016; Schmitz et al., 2017).

Meanwhile, sexual dimorphism in the human brain remains complicated. The mean weight of the male and female brains has been shown to be 1350 g and 1250 g, respectively (Roland & Zilles, 1994). In line with post-mortem data, more recent *in vivo* studies of sex differences in the adolescent brain volume have shown that males tend to have significantly larger brains than females by approximately 9–12% due to average body size differences (Allen et al., 2002; Lenroot & Giedd, 2010; Ruigrok et al., 2014). Some speculations about cortical thickness and cognitive differences regarding sex occurred, suggesting that factors driving the development of typical sex differences might also play a role in neuropsychiatric conditions (Ruigrok et al., 2014). Ruigrok et al. (2014) suggested candidate regions for investigating the asymmetric effect that sex has on the developing brain to understand sex-biased neurological and psychiatric conditions; the main ones are the amygdala, hippocampus and insula. However, besides already introduced facts of sex differences in brain volume and morphology (Allen et al., 2002; Lenroot & Giedd, 2010; Ruigrok et al., 2014), the grey/white matter ratio (Gur et al., 1999) and structural asymmetry (Kong et al., 2018), other observations regarding sex are quite speculative (Cahill, 2006, 2012; McCarthy et al., 2012).

A similar claim is relevant and for age-related differences in human brain macrostructure anatomy. The overall trend of brain shrinkage with age is thought to be in a linear fashion and was also observed in Kong et al.'s (2018) meta-analysis: significant age effects were only observed with those datasets with broader age ranges (at least a 20-year range). Specifically, increasing age was associated with more pronounced leftward overall asymmetry in cortical thickness, which partly reflects a similar age effect on the thickness asymmetry of the superior temporal gyrus. Age has been found to be a significant moderator in cognitive performance between sexes, i.e. indicating that sex differences in visual-spatial working memory were apparent in teenagers but not older groups (Voyer, Voyer, & Saint-Aubin, 2016). This corresponds to larger effects of sex (females > males) in the younger samples than the older samples in a structural meta-analysis. However, age-related structural asymmetries are still not as apparent as sex-related: the frontal (superior frontal gyrus, the pars orbitalis region of the left

inferior frontal gyrus), temporal (superior temporal gyrus, temporal pole, parahippocampal gyrus, and fusiform gyrus), parietal (inferior parietal gyrus and supramarginal gyrus) and anterior cingulate cortices (Figure 1.3.2).

Finally, it is interesting to note that Kong et al.'s (2018) meta-analysis found several significant and replicable brain asymmetries with two independent pedigree datasets (n = 1,443 and 1,113 respectively). These datasets were used to estimate heritability of the asymmetry measures, and the study results indicated that most genetic effects on structural variation are shared bilaterally, but some independent genetic effects exist in each hemisphere. These findings may support future studies on the genetic basis of brain asymmetry and altered laterality (regarding neurological and psychiatric disorders) and potentially may also be associated with inter-hemispheric differences in gene expression, which in turn could help to better understand whether handedness is indirectly linked to brain structure (Francks, 2015; Karlebach & Francks, 2015; Sun & Walsh, 2006).

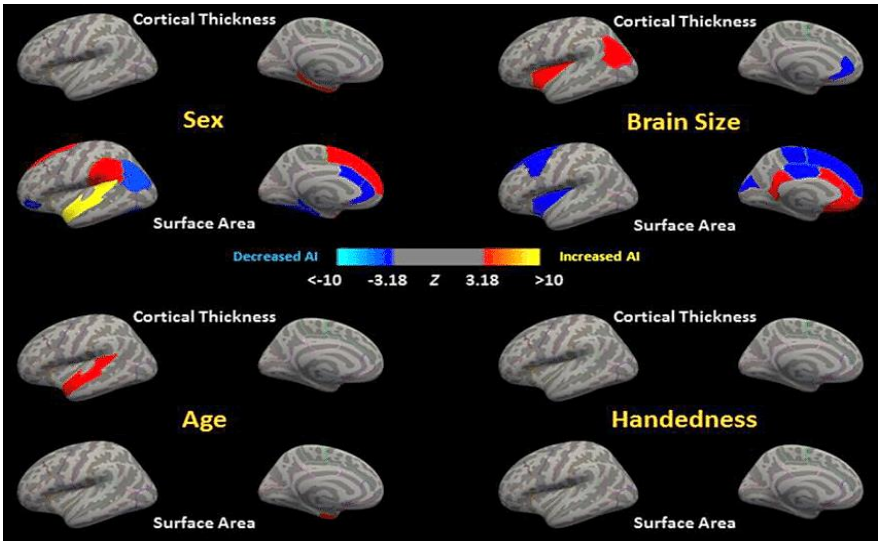

Figure 1.3.2. Meta-analysis results for the effects of sex, age, handedness and ICV on regional asymmetry indexes in cortical thickness and surface area. Red–yellow indicates an increased asymmetry index (AI) in males/ with age and ICV; blue–light blue indicates a decreased AI in males/with age and ICV. AI was defined as (L - R)/((L + R)/2). And z-threshold of 3.18 (p = 0.05, Bonferroni corrected) was used. For more details, see *Datasets S3–S6.* ICV is an important normalisation measure used in morphometric analyses to correct for head size (Kong et al., 2018).

Sexual dimorphism and cognitive sex differences

Sexual dimorphism refers not only to differences in morphology, but also sex differences in physiology and behaviour (McCarthy et al., 2012). Some most often defined sex-related differences in cognition are in the verbal and spatial domains, where females dominate in verbal and men in spatial ability tasks (Bolla, Eldreth, Matochik, & Cadet, 2004; Dong, Guo, Zhang, Fu, & Shi, 2010; Goldstein et al., 2005; Gur et al., 1999; Mowrey & Portman, 2012; Nowak, Resnick, Elkins, & Moffat, 2006; Overman, 2004; Sacher et al., 2013); Some relate these differences to working memory (Hill et al., 2014; Voyer et al., 2016). Many other researchers naturally link it to the sex hormones, such as estrogen and testosterone, known to impact sexual dimorphism in the brain (Bale & Epperson, 2017; Becker et al., 2005; Cosgrove, Mazure, & Staley, 2007; Hausmann, 2017; Lenroot & Giedd, 2010; Short, Yang, & Jenkins, 2013). Unfortunately, this knowledge is often used in favour of the researcher's goals, as the majority of research has focused on expectancy to detect sex differences in relation to test performance (Hirnstein, Coloma Andrews, & Hausmann, 2014; Miller & Halpern, 2014). Or on the other end of the spectrum, it is also sometimes used to support insufficient experimental design based on male participants only (resulting in sex bias when the effects from males are generalised for females as well (Check Hayden, 2010; McCarthy et al., 2012).

There are several reasons sexual dimorphism in the human brain and cognitive sex differences are so important to adequately define and why it is so difficult. First, distinctions between organisational and activational effects of sex hormones are often difficult to distinguish empirically, especially because environmental experiences continue to shape brain structure even in adulthood (Miller & Halpern, 2014; Overman, 2004). Second, cognitive sex stereotyping related to cultural influences continues to bias researchers even though there is sufficient scientific evidence that males do not necessarily outperform females in mathematical and spatial abilities, that females' advantages in verbalization are not absolute, and that both depend on a given task (especially considering cross-national trends) (Miller & Halpern, 2014; Stoet & Geary, 2013; Voyer et al., 2016). This is thought to be related to sexual dimorphism in the brain functions regarding working memory. For example, the meta-analysis of working memory abilities during several cognitive tasks demonstrated consistent working memory networks across genders, but also

provided evidence for gender-specific networks whereby females consistently activate more limbic (e.g. the amygdala and hippocampus) and prefrontal structures (e.g. the right inferior frontal gyrus), and males activate a distributed network inclusive of more parietal regions (Hill et al., 2014). Third, psychophysiological human studies mainly rely on using non-invasive functional neuroimaging approaches, such as fNIRS and fMRI, where not all technique-related issues with accurate translation of physiological signals into neuroimaging data are currently addressed (Tachtsidis & Scholkmann, 2016; Toga, 2015). Finally, the overall inheritability of brain structural and functional architecture remains ambiguous (Elliott et al., 2018). For example, we still know very little about why there are sex-related differences and odd ratios in handedness. According to Papadatou-Pastou et al.'s (2008) meta-analysis of 144 studies and 1,787,629 subjects, men are more likely to be strong dominant left-handers than women (odds ratio is 1.23), and men are also more prone to ambidexterity (Papadatou-Pastou et al., 2008).

Handedness and predominant hemispheric lateralisation

The most frequently recognised manifestation of functional lateralisation is the dominance of the left hemisphere for handedness and language (Corballis, 2014; Häberling, Corballis, & Corballis, 2016; Toga et al., 2009). Both functions, preferred hand control and language, are located in the frontal cortex, and yet is not completely clear what its impact is on the neuroimaging results of the tests involving other higher executive functions (Badre & D'Esposito, 2009; Häberling et al., 2016; Habib, Nyberg, & Tulving, 2003; Hervé et al., 2013). Interestingly, language dominance and handedness are somehow linked in brain architecture, possibly due to evolution, but not perfectly correlated. Approximately 97% of right-handers have their speech and language localised in the left hemisphere, whereas 3% demonstrate right hemisphere lateralisation or bilateral language representation. These relationships degrade to 70% (right-lateralized) versus 30% (left-lateralized) in left-handed individuals, making the left hemisphere dominant for both tasks for more than 90% of the population (Hervé et al., 2013; Knecht et al., 2000; Toga et al., 2009). This makes handedness a behavioural marker for language lateralisation in the brain and attracts significant research interest.

Moreover, familial sinistrality (Hervé et al., 2013; Mellet et al., 2014; Papadatou-Pastou et al., 2008) together with the degree of handedness has been shown to be a promising measure of brain laterality in some cognitive

studies (Corballis, 2014; Hervé et al., 2013; Vuoksimaa, Koskenvuo, Rose, & Kaprio, 2009). Although specific gene products have proven to mediate the development of brain and body asymmetry, the real genetic role thereof in hand preference remains ambiguous (Brandler & Paracchini, 2014; Corballis, 2014; Robinson et al., 2016; Schmitz et al., 2017). As a result, right-handed bias in neuroscience research appeared, when some researchers *a priori* or *a posteriori* excluded left-handers from their analysis after observing some inconsistencies (Willems, Van Der Haegen, Fisher, & Francks, 2014). Despite the complexity of the explanation of handedness involvement with other factors, it is critical to account for this variation in our understanding of brain functioning. This is mainly because left-handed subjects represent a substantial portion of the human population and therefore fall within the normal range of human diversity (Schmitz et al., 2017; Willems et al., 2014).

1.3.3. The WCST

The WCST is a neuropsychological tool first introduced by Grant and Berg in 1948 (Berg, 1948; Grant & Berg, 1948). The test was originally developed for use with patients suffering from brain disorders affecting the frontal lobes (Grant & Berg, 1948). For decades, it has been one of the most distinctive tests of prefrontal dysfunction in patients with acquired brain injury, neurodegenerative disease or mental illness (Eling, Derckx, & Maes, 2008; Heaton, 1981; Milner, 1963; Nyhus & Barceló, 2009)

The WCST is generally regarded as the prototype abstract reasoning task and has been routinely used to assess frontal lobe function in clinical and research contexts. It is also known, and sometimes referred to, as the Berg Card Sorting Test (BCST). Historically, these two names are used interchangeably because the first name, created after the name of the university, soon became the trademark. Thus, second name is often used to honour the founder of the test and avoid legal issues. Like the commercially sold WCST, the standard version in the freely available test batteries, the BCST consists of 128 cards, based on the same procedures originally described by Berg (Berg, 1948; Grant & Berg, 1948). Currently, there are at least two different systems of administration and scoring of the WCST: the standard version by Grant and Berg (1948), (incorporating Milner´s (1963) correction criteria in scoring system) and shortened versions by Heaton (1981).

In the standard version of the WCST, participants are required to use their performance feedback to determine and shift between different card sorting rules with respect to shape (crosses, circles, triangles or stars), colour (red, blue, yellow or green), or the number of figures (one, two, three or four), (Figure 1.3.3). The participant is not instructed as to which of the three criteria to use; thus, it requires reasoning and the use of given feedback on a trial to determine the correct criteria. The criteria changes without warning, and by this constantly requires the participant to update her/his mental representation. There are several types of error reports by which participants' performance is scored: the total number of errors, perseveration errors (when you keep applying the old rule of sorting) and non-perseveration errors, or the total percentage of correct sorting. The test can be presented manually, or lately more often in a computerised version, but both consist of the same structure (Figure 1.3.3). The freely accessible digital BCST (v0.6) from the Psychology Experiment Building Language (PEBL v0.13) test battery that was used for experiments (Mueller & Piper, 2014) follows the same rules and structure of administration as the widely known WCST (Piper et al., 2012); the more well-known name of the WCST was chosen to be used for this dissertation for clarity and consistency.

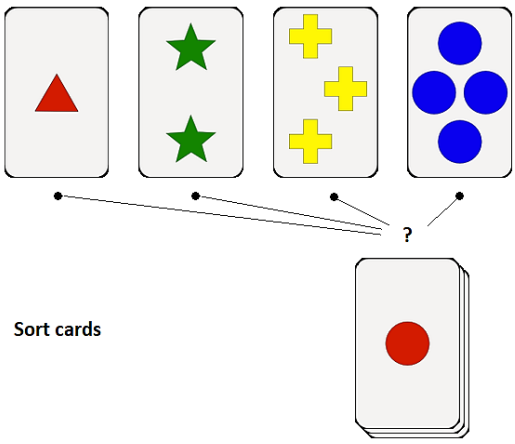

Figure 1.3.3. The WCST.

Clinical implications

Numerous clinical studies on WCST performance have reported impairment on the WCST with frontal cortex damage. Because the WCST evaluates the essential ability to shift mental sets as well as update and monitor working

memory representations, the most common applications are regarding traumatic brain injury, epilepsy, tumours, strokes and several psychiatric disorders, including schizophrenia (Nyhus & Barceló, 2009).

However, despite a quite rich history in clinical applications and research, there is no clear and consistent link between the location of the lesion and the behavioural results on the WCST: there have been reports that left frontal damage affects WCST performance more than right frontal damage (Goldstein, Obrzut, John, Ledakis, & Armstrong, 2004), while the meta-analysis reported no such difference in the laterality of damage in the frontal cortex (Demakis, 2003). Although researchers have investigated potential functional laterality, it is likely that the WCST does not distinguish between left and right damage of the PCF. Moreover, several clinical studies show that damage in non-frontal or diffuse damage in frontal and non-frontal regions cause similar impairments to WCST performance as those subsequent to frontal lobe lesions (Nyhus & Barceló, 2009). However, despite these concerns, clinicians still routinely use the WCST to accommodate their clinical assessment. Functional neuroimaging is not that common due to a lack of specificity, as briefly overviewed across different health conditions and neuroimaging modalities by Nyhus and Barceló (2009).

Functional neuroimaging

Most of functional neuroimaging studies on WCST performance have been focused on groups of patients versus healthy participants. The main reason is because in principle, normal subjects were thought to show a more homogeneous level of behavioural performance than clinical samples and, consequently, their functional brain imaging results were expected to show better anatomical consistency and specificity than lesion studies (Nyhus & Barceló, 2009).

For healthy subjects alone, the WCST is used to measure an individual's competence in abstract reasoning, and the ability to change problem-solving strategies when needed and thought is considered a useful task for cognitive evaluation (Kane & Engle, 2002; Nyhus & Barceló, 2009). Most studies of healthy participants reveal activation in a widespread neural network of prefrontal, frontal, temporal, parieto-temporal and parieto-occipital cortical regions during various stages of WCST performance (Nyhus & Barceló, 2009). Only a few fMRI studies have examined further. For example, a quantitative fMRI meta-analysis found extensive bilateral clusters of reliable cross-study activity in the lateral prefrontal cortex, anterior

cingulate cortex and inferior parietal lobule. In the same study, it was indicated that task switching in particular reveals a similar, although less robust, frontoparietal pattern with additional clusters of bilateral activity in the opercular region of the ventral prefrontal cortex (Buchsbaum, Greer, Chang, & Berman, 2005). It corresponds to another study of healthy participants, which used a different task complexity to decompose the neural processes underlying the WCST. The results indicated that the right ventrolateral prefrontal cortex was related to simple working memory operations, while right dorsolateral prefrontal cortex was related to more complex/manipulative working memory operations. The rostral anterior cingulate cortex and the temporoparietal junction bilaterally represented an attentional network for error detection. In contrast, activation of the caudal anterior cingulate cortex and the right dorsolateral prefrontal cortex was associated with increased attentional control in the context of increasing demands of working memory and cognitive control (Lie, Specht, Marshall, & Fink, 2006). These findings partially explain why, for example, compared with other tests for executive functioning, such as the Iowa Gambling task, patients with a damaged centre on the ventromedial prefrontal cortex were worse during gambling, but performed normally on the WCST (Overman, 2004). This also raises the question of why the effect of sex in the WCST was not previously explored, as the same study by Overman (2004) found sex differences on somewhat a similar decision-making task: the Iowa Gambling Task. Another study on executive functioning focused on ventromedial prefrontal cortex lesion also found a systematic effect of sex on the pattern of left–right asymmetry (Tranel, Damasio, Denburg, & Bechara, 2005).

Sex, handedness and fNIRS

Sex differences in cognition are of great interest but are often the result of a confusing mixture of psychological, physiological and cultural factors (Hirnstein et al., 2014; Miller & Halpern, 2014; Voyer et al., 2016). Similarly, handedness has long been regarded as a behavioural marker of functional asymmetry, but it is not clear how (Badre & D'Esposito, 2009; Häberling et al., 2016; Habib et al., 2003; Hervé et al., 2013). However, a review of the literature on sex and handedness effects regarding the WCST gave no particular results: there were no extensive investigations on sex and handedness effects using functional neuroimaging. Thus, sex or handedness have never been associated with the WCST performance (Eling et al., 2008; Nyhus & Barceló, 2009; Overman, 2004).

Because the composition of participant groups within and across studies vary significantly, it can be hypothesised that sex and handedness might be a confounding factor of the apparent inconsistency in functional neuroimaging results of the WCST. The variation in the composition of groups can be, for example, clearly seen in a previously discussed overview by Nyhus and Barceló (2009; Table 1-2). Further, this hypothesis can be supported by other recent functional neuroimaging research using fMRI, which demonstrated that individual variability should be thoroughly addressed before population-level inferences (Bidula et al., 2017; Finn et al., 2015; Friedman & Miyake, 2017). Thus, since no similar investigation of sex and handedness effects on the WCST functional neuroimaging results exists, the possibility of the previously discussed hormonal influence on the HR together with handedness-associated functional lateralization cannot be neglected.

Another important aspect that gave rise to the second research project presented in the dissertation is that only a few previous studies have used fNIRS for investigating the WCST (Fallgatter & Strik, 1998; Hashimoto et al., 2007; Sumitani et al., 2006), and they all demonstrate inconsistent findings as reported on other neuroimaging techniques reviewed by Nyhus and Barceló (2009). The overview does not include fNIRS, likely because of the same reason – a rare application of fNIRS. However, the reason there are so few studies with fNIRS is not specific to the WCST – this functional neuroimaging technique only quite recently gained attention in the neuroimaging community (Boas et al., 2014). Meanwhile, existing studies with fNIRS were carried out in small and heterogeneous groups of healthy participants or patients. None studies proposed a reasonable explanation of their contradicting findings. Fallgatter and Strik (1998) and Hashimoto et al. (2007) had a low spatial resolution (2 optodes) or one region of interest per hemisphere. Thus, their conclusions are restricted to the right hemisphere being more active than the left in healthy controls. Sumitani et al.'s (2006) study had a higher spatial resolution (24 optodes) and proposed different patterns of activation in the PFC among healthy subjects, but did not provide a consistent and reasonable explanation of their findings. In contrast to other techniques, fNIRS is a convenient and robust method for such investigation.

Moreover, it is also hypothesised that if sex and handedness has any effects on the HR evoked in the prefrontal cortex, the procedure for how fNIRS data is prepared for a statistical inference might potentially affect the final results. Thus the statistical data analysis should be performed with two datasets driven from the same raw data collection: the first dataset, pre-

processed data, would refer to commonly used fNIRS data processing when only cleaning of noise (pre-processing) is performed; the second dataset, normalised data, would refer to a simple normalisation procedure, performed right after pre-processing. The intention of normalisation is that normalised values allow the comparison of corresponding normalised values for different datasets to eliminate the effects of certain gross influences. Since sex hormones in general could potentially be more influential on HR parameters than functional laterality associated with handedness, the normalisation would adjust the systematic hormonal influence and help to reveal handedness-related differences between the same sex groups. At the same time, if sex effects are obtained on the pre-processed-only dataset, but can be eliminated by the normalisation, it would mean that original data distribution is a mix of two overlapping distributions and would re-confirm the existence of sex-related effects. In addition, it is unclear whether sex-related odd ratios for left-handers could reflect on functional laterality during the WCST performance. By this, normalisation in the presence of sex effects might be the only way to capture such interaction.

In sum, the consistent investigation of sex and handedness on the functional neuroimaging results of healthy participants would provide new insights not only in basic research but also on clinical implications. First, if there are no sex or handedness effects on the functional neuroimaging results of the WCST performance, it would strengthen the population-level inferences from the studies that investigate sex-biased conditions, such as autism or schizophrenia. Second, if there are sex or handedness effects on the functional neuroimaging results of the WCST performance, it would be a strong reason to suggest re-evaluating existing studies by taking into account the composition of patient versus healthy participant groups, and could in turn potentially help to at least partially explain the existing inconsistencies in WCST results.

2. MATERIALS AND METHODS

2.1. Developing a CW-fNIRS system for human frontal lobe studies

2.1.1. The fNIRS system for human frontal lobe studies

The goal was to design a CW-fNIRS-type spectroscopic system that would be suitable for frontal lobe studies in human adults, easily replicated and rearranged for specific needs. To meet this goal, a manually constructed system was designed to be easily upgraded from one or two optodes in the early stage to a multichannel probe. To achieve this, the system was built of the following:

1) a semi-flexible forehead sensor;

2) a sensor control unit, for synchronisation and initial data processing;

3) a data acquisition module (DAQ) for further data processing;

4) a laptop computer with data acquisition and calculation software.

The developed sensor consisted of two LED light sources and two detectors, with a source-detector separation of 2.5 cm. The light sources were chosen to at 735 nm and 850 nm wavelengths. As a photodetector, a monolithic photodiode with a single supply transimpedance amplifier was used (OPT101). As a background, polymeric clay was used, which had a steady structure, but remained flexible as well. Thus, the sensor was sufficiently flexible on the subject's head, but optodes remained immobilized. The sensor control unit ensured the synchronization between emitters and the detectors. The initial data recordings were sent to a computer through a DAQ (see Figure 2.1.1 A). The simplified block diagram in Figure 2.1.1 A demonstrates the scheme of how one channel is synchronised to be sampled at 300 μs interval. The overall manually designed system's sampling rate was 100 Hz.

To validate the designed system, it was compared with a commercial CW-type 16 optode device fNIR 400 from Biopac. This sensor has four light sources and 10 detectors with the same source-detector separation of 2.5 cm (see Figure 2.1.1. B). It also uses similar LED wavelengths of 730 nm and 850 nm. Due to the differences in sensor geometry, the comparison of fNIR 400 and manually designed system was made regarding optodes: the 1^{st} and 2^{nd} (see Figure 2.1.1. C), which approximately corresponded to the 5^{th}, 6^{th} and 11^{th}

and 12th optodes in the fNIR 400 probe (see Figure 2.1.1 B). The overall fNIR 400 system's sampling rate was 2 Hz.

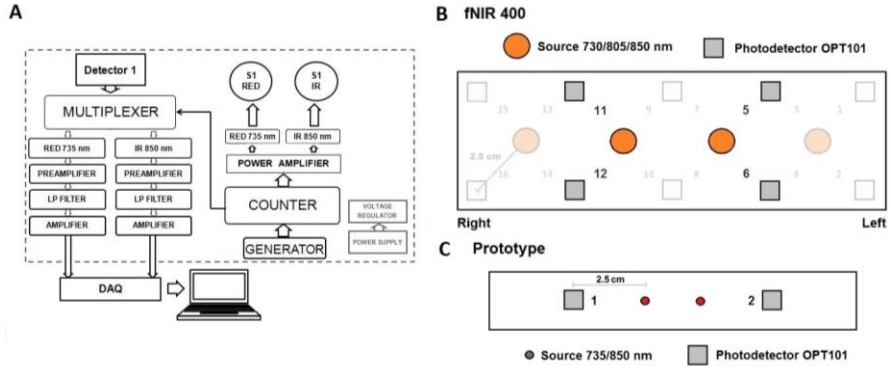

Figure 2.1.1. (A) A simplified block diagram of one optode sensor control unit. One light source consisted of two wavelengths (850 IR and 735 RED). By the counter, they were turned on one-by-one in a sequence. At the same time, a counter triggered a multiplexer to sample incoming light with a detector. Both separate channels were amplified, filtered from noise and transmitted to DAQ. DAQ-data acquisition module; S1-Source number 1; LP-low-pass filter. **(B)** The probe configuration of the fNIR 400 device and **(C)** the manually designed system. Sensor geometry and central alignment was made with accordance to the 10-20 EEG system. The comparable optodes between devices are indicated.

2.1.2. Computerised WCST

To validate that the manually designed system is able to record the evoked by the task HR, the experiments were performed with the WCST, which was thought to be a standard test for frontal lobe activity. The WCST was administered to all subjects using the freely accessible digital WCST (v0.5) from the Psychology Experiment Building Language (PEBL, v0.12) test battery (Mueller & Piper, 2014).
Validation tests were performed using the same protocol for both devices and consisted of these main steps:

a) Introduction to the task and basic instructions;
b) Recording of a baseline with closed eyes in a calm-posture position (-60 sec for the designed system and -10 sec for fNIR 400);

c) Recording the WCTS performance (~300 s).

The proposed baseline length differs because of the built-in duration of fNIR 400 baseline recording and the decision to have a longer baseline for the manually designed system to ensure participants' comfortability with a probe before the main experiment. During the recording of the WCST, the participant's performance time was marked individually. The rest of time the participant was asked to remain calm and keep his/her eyes closed until the experiment ended to additionally capture the reverse of chromophores concentration after the presence of a mental load.

Due to the differences in sensor geometry, from all 16 optodes in fNIR 400, only the 11^{th} and 12^{th} optodes according to system optode 1 (right hemisphere) and the 5^{th} and 6^{th} optodes corresponding to optode 2 in a manually developed probe (left hemisphere) were included for further analysis (Figure 2.1.1 B).

2.1.3. Subjects

All participants were recruited from a university using a poster advertising. Informed written consent was obtained from each participant before inclusion and the study protocol was approved by the local ethics committee. The validation study was performed with eight 22 ± 2-year-old right-handed male subjects for the manually developed system and repeated with eleven 24 ± 3-year-old right-handed male subjects for fNIR 400. Handedness was assessed using the Flinders Handedness Survey (Nicholls, Thomas, Loetscher, & Grimshaw, 2013) (Supp.1).

2.1.4. Signal pre-processing and data comparison for validation

Signals coming from registering photodetectors in the manually developed system were separated into two channels and analogically filtered with a low pass filter (∽10 Hz), later amplified and sent to the computer for further analysis. The MBLL was applied on obtained data manually using MATLAB (The MathWorks Inc., MA, USA), and graphics were made with Origin Pro (OriginLab Corp,MA, USA). For optical data acquisition using fNIR 400, the Cognitive Optical Brain Imaging (COBI) Studio software (v1.3), (Drexel University) and for data analysis fnirSoft (Ayaz, 2010) (version 3.5,

fNIR Devices, USA) were used. Statistical analysis was carried out using a two-sample t-test.

2.2. Investigation of sex and handedness effects on the WCST evoked the PFC HR

2.2.1. Subjects

All participants were recruited from a university using poster advertising. Informed written consent was obtained from each participant before inclusion. The study protocol was approved by the local ethics committee. In total, 18 females in their follicular phase and 17 females in their luteal phase were included with an equal ratio of right-handers to left-handers. Menstrual phases were established using questionnaires and described according to the classical menstrual cycle (Hausmann, 2017). The participants declared no use of contraception or major irregularities in their cycle. In the early investigation stage, the additional analysis on the menstrual cycle of females gave no particular results alone or in relation with task performance (Razinskaite & Cinciute, 2017). Thus women participants subsequently were treated as one group regardless of menstrual phase. Handedness was assessed using the Flinders Handedness Survey (Nicholls et al., 2013),(Supp.1). Ultimately, the cohort of 70 subjects was collected (female/male, n = 35/35; right-handed/left-handed, n = 40/30), (Table 2.2.1).

Table 2.2.1 The composition of participant groups by sex and handedness.

Sex	**Female**		**Male**	
Handedness	**Right**	**Left**	**Right**	**Left**
Number of subjects	20	15	20	15
Age, mean ± SD	21.45 ± 0.89	21.93 ± 3.49	20.55 ± 2.30	22.08 ± 3.56
FLANDERS score, mean ± SD	9.55 ± 0.89	-9.73 ± 0.80	9.65 ± 0.93	-8.79 ± 1.92

2.2.2. Computerised WCST

The WCST was administered to all subjects using the freely accessible digital WCST (v0.6) from the Psychology Experiment Building Language (PEBL v0.13) test battery (Mueller & Piper, 2014). The original full-length test was modified such that subjects had to complete four blocks: an example block consisting of 24 cards (three sorting rules); two identical Modified Comparison (MC) blocks before and after the main WCST composed of 64

cards and a no-rule switch; the main block with the standard WCST consisting of 128 cards and all three sorting rules, as originally found in the battery. Between each block, 30 sec of rest were included (Figure 2.2.1). The test modifications were made to control for possible non-executive frontal modulation related to the stimulation and verify the sensitivity of fNIRS for task-related frontal lobe activity. Together, the MC blocks serve as evaluation periods to assess whether there are any detectable differences in cognitive strategies or neurovascular dynamics before and after subjects learn the WCST. Oral and written instructions for the experiment and WCST were provided: subjects were requested to use their performance feedback to find the correct classification principle by trial and error for all cards with geometric figures that vary according to three perceptual dimensions (colour, symbol or number). There was no time limit to finish the test. The whole experiment was recorded in a single individual session. The hypothesis was that the mean HR parameters would be highest for the WCST block, whereas values for the second MC period would be lower than the first MC period, demonstrating underlying adaptation mechanisms.

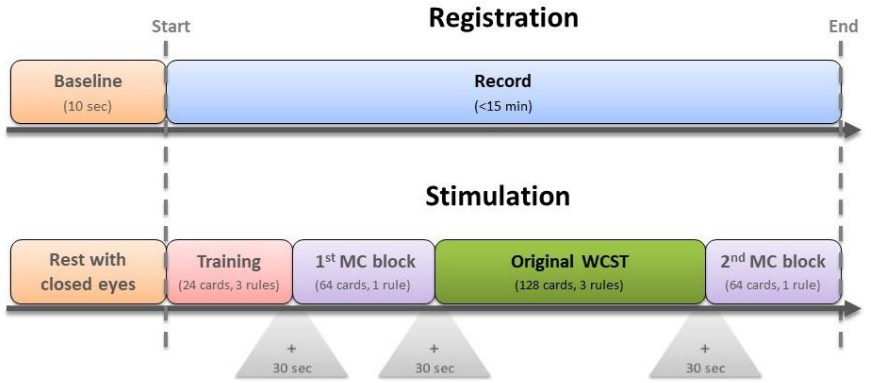

Figure 2.2.1. Schematic representation of the data collection. The upper part demonstrates a recording of a single individual session after a mandatory 10-second baseline. The lower part demonstrates the experimental design, where two identical MC blocks before and after the main block of original WCST were present. Each block was separated by a 30 sec period of fixation at the cross.

2.2.3. fNIRS recording system

The study was conducted using a continuous-type 16 optodes (up to 48 channels)[1] Device fNIR 400 from Biopac, USA (Figure 2.2.2). The sensor is designed to monitor rostral cortical areas beneath the forehead, including Brodmann areas 10, 9, 45 and 46 (Ayaz et al., 2010; Izzetoglu et al., 2007). It has a 2.5 cm source-detector separation allowing for approximately 1.25 cm penetration in depth and a temporal resolution of 2 Hz. Measurement values are in micromolar units (μMol/L), in contrast with a pre-recorded 10-second baseline. The probe was aligned with the electrode positions FP1 and FP2 based on the international 10–20 EEG system (Ayaz et al., 2006). For optical data acquisition, the Cognitive Optical Brain Imaging (COBI) Studio software (v1.3, Drexel University) was used.

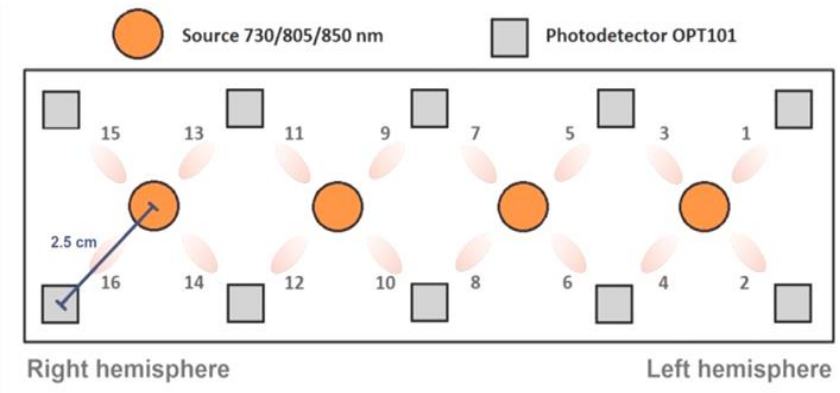

Figure 2.2.2. Probe configuration of the fNIR 400 device. Ellipses show a graphical approximation of measurement locations (Venclove et al., 2015).

The device can represent the obtained HR in three ways: as a temporal plot, as bars of concentrations or as a functional map on the standard human forehead template. In neuroimaging, functional mapping usually implies mapping function into an anatomical space, e.g. using statistical parametric mapping. In this specific case, regarding the fNIR 400, the HR functional maps are the spatial interpolation of the local concentrations across the optodes. The initial concentrations are obtained in particular positions

[1] Optode is a term in spectroscopy to name a pair of constituted by an emitter and a photodetector. As multiple wavelengths may be used in the emitter, one optode may form from two to several channels.

between the light source and the detector (which are indicated as ellipses in Figure 2.2.2). These are later interpolated within a conceptual voxel with the common interpolations method on the researcher's selection (Ayaz et al., 2006). Measurement values correspond to what was interpolated and are in micromolar units (μMol/L) if not indicated otherwise.

2.2.4. Preprocessing of fNIRS signals

The fNIRS signals were pre-processed as described: (a) a visual inspection of raw data quality was performed; (b) a low-pass finite impulse response (FIR) filter (order 20) with the cut-off frequency set to 0.1 Hz (Izzetoglu, Izzetoglu, et al., 2005) was used to eliminate respiration and heart pulsation artefacts; (c) the sliding-window motion artefact rejection (SMAR) algorithm was used (window size 10, threshold range from 3 to 25,(Ayaz et al., 2010)); (d) the MBLL (Cope & Delpy, 1988) was implemented to calculate the following hemodynamic parameters: change in the HbO_2, HbR, total blood volume (HbT, the sum of HbO_2 and HbR) and oxygenation (OXY, the difference between HbO_2 and HbR). Data pre-processing, the calculation of the HR parameters and topographic representations were performed using fnirSoft (Ayaz, 2010),(version 3.5, fNIR Devices, USA).

2.2.5. Approaches used for fNIRS data investigation

The fNIRS community still does not have unified recommendations on how fNIRS data should be processed. Bearing this in mind, this study intentionally avoids the overuse of complicated data cleaning, processing and analysis procedures to ensure that the final results are not due to the interference of these factors and only correspond to the obtained signal.

The first dataset, pre-processed data, refer to commonly used fNIRS data processing when no normalisation or standardisation is done. This dataset will subsequently be referred to as pre-processed data.

The second dataset, normalised data, refers to a simple normalisation procedure, performed right after pre-processing dividing signal by the maximum value:

$$X = \frac{x_i}{|max\ x_i|};$$

where x_i is a signal and $max\, x_i$ is a maximal value of a signal obtained in optode number *i*. Subjects performed the WCST at their own pace before the group analysis data were first rescaled in time into a range of [0,1].

Data for all 70 subjects are separated according to four groups (right- and left-handed females, and right- and left-handed males). Table 2.2.1 shows the number of subjects, mean and standard deviations of age, and handedness scores. Figure 2.2.3 provides a better explanation of the data preparation and statistical analysis workflow.

2.2.6. Statistical data analysis

WCST. Two-way analyses of variance (ANOVAs) were used to examine the sex and handedness effects on the number of perseveration errors, correct test sorting rate and total duration of WCST performance.

fNIRS data. First, two-way repeated measures multivariate analyses of variance (RM-MANOVAs) were conducted to explore the effects of sex and handedness on multiple HR parameters ('HbO_2' and 'HbR'; 'HbT' and 'OXY') that have repeatedly been assessed for three-time point ('1st MD block', 'WCST' and '2nd MD block'). Tests were conducted with two pairs of HR measures ('HbO_2' and 'HbR'; 'HbT' and 'OXY'), as HbT and OXY are linear combinations of HbO_2 and HbR and were highly correlated ($r > 0.8$). Therefore, these pairs were examined separately. The previously described procedure was performed in parallel for both datasets (pre-processed and normalised). Tests were carried out with Bonferroni adjustments ($\alpha = 0.05$) for multiple comparison corrections where necessary. To better explain the workflow of the fNIRS data analysis, it is graphically presented in Figure 2.2.3.

Second, the effects of sex and handedness on the HR parameters (HbO_2, HbR, HbT and OXY) in 16 optodes were explored by conducting RM-MANOVAs on the WCST block. As before, pairs of HR parameters were examined separately. In the analysis, the within-subject factor was defined as 'Optode' (with 16 dependent variables per measure). The alpha level was set at 0.05. Wilks' lambdas were used to test whether there are differences between the means of independent groups of subjects on a combination of dependent variables. Effect sizes were examined as the proportion of variance accounted for by partial η^2. Whenever the sphericity assumption was violated, Greenhouse-Geisser corrected values were used for further analysis. Tests

were carried out with Bonferroni adjustments ($\alpha = 0.05$) for multiple comparison corrections where necessary.

The third analysis was similar, but it was conducted with a focus on hemispheric differences and the sex and handedness effects on the HR parameters (HbO_2, HbR, HbT and OXY). The RM-MANOVA on the WCST block was conducted where the within-subject factor was defined as 'Hemisphere' (with eight dependent variables per measure). Pairs of HR parameters were examined separately and calculations repeated for both datasets. The alpha level was set at 0.05. Wilks' lambdas were used to test whether there are differences between the means of independent groups of subjects on a combination of dependent variables. Effect sizes were examined as the proportion of variance accounted for by partial η^2. Whenever the sphericity assumption was violated, Greenhouse-Geisser corrected values were used for further analysis. Tests were carried out with Bonferroni adjustments ($\alpha = 0.05$) for multiple comparison corrections where necessary.

2.2.7. Post-hoc data analysis

Concerning the question of which optodes are significantly different among the discrete groups of subjects, a *post hoc* one-way ANOVA was conducted. A single independent factor analysed 16 dependent variables, 'Group', (right-handed females, left-handed females, right-handed males and left-handed males; see Table 2.2.1). A multiple comparison correction was carried out with Bonferroni adjustments ($\alpha = 0.05$). Additionally, a paired-sample t-test was applied when necessary to investigate the directionality of the obtained effects.

Additionally, the laterality index (LI) was used to investigate hemispheric dominance and to compare the results with previous fNIRS studies. Hemispheric specialisation was defined by the LI using the formula $LI = (L - R)/(L + R)$, where L and R indicate the sum of the mean values of the concentrations on the left and right hemispheres, respectively: the first eight optodes (1–8) were averaged to represent the left hemisphere, while the last eight optodes (9–16) were averaged to represent the right hemisphere (Figure 2.2.3). A positive LI value indicates left-hemisphere dominance, and a negative value indicates right-hemisphere dominance.

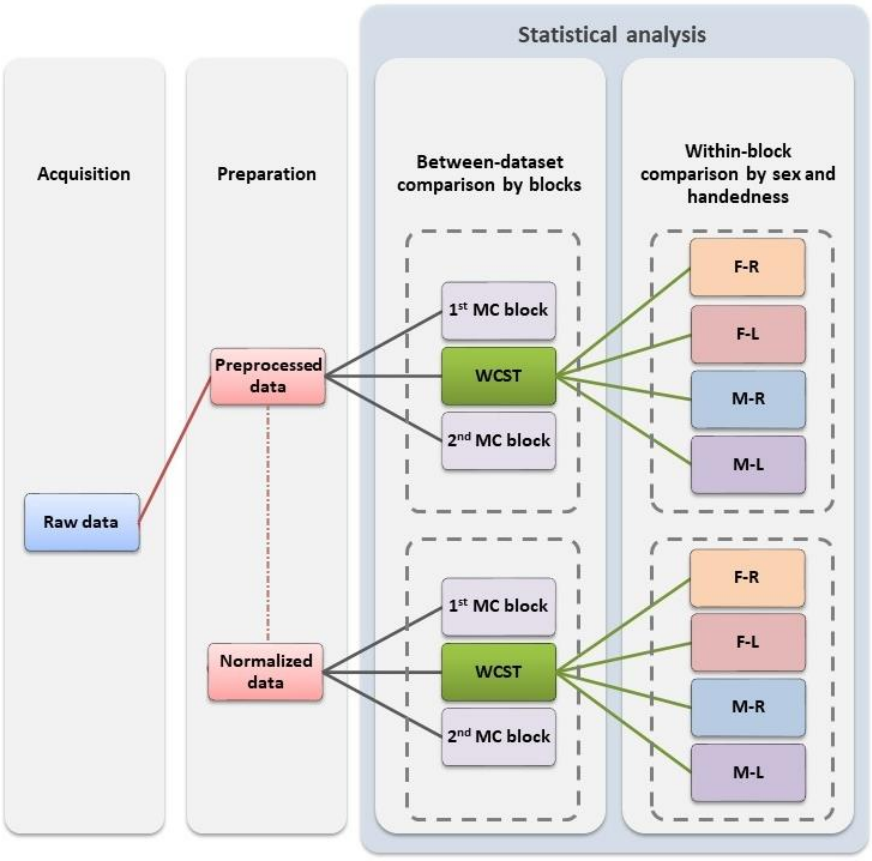

Figure 2.2.3. Workflow of fNIRS data preparation and statistical analysis. After the acquisition, on the preparation stage, the raw data were used to form two alternative datasets: pre-processed (formed after the necessary data cleaning procedures) and normalised (composed after basic data cleaning procedures and normalisation by the maximum value). A between-dataset comparison (by experimental blocks, subsequently grouped by sex and handedness) was performed in parallel for both datasets. A further detailed within-block comparison (for each HR parameter and grouped by sex and handedness (F– female; M– male; R– right-handers; L– left-handers)) was made. Modified Comparison (MC) block; Wisconsin Card Sorting Test (WCST).

3. RESULTS

3.1. Developing a CW-fNIRS system for human frontal lobe studies

3.1.1. WCST

All participants showed normal performance during the WCST: no strong individual differences in the duration of task performance or regarding the test results were found. The mean number of perseveration errors was 13.25 ± 5.50 (99% confidence interval = 10.00, 16.45), and the mean correct sorting rate was 80.06 ± 6.82% (99% confidence interval = 76.58, 84.62).

3.1.2. The manually developed cw-fNIRS system

A paired-sample t-test revealed that the parameters of HR HbO_2 and HbR differ significantly ($t(7) = 2.36$, $p = 0.02$) and can be seen as evidence for real signal detection. The strong negative correlation between the recorded HbO_2 and HbR signals ($r > -0.90$) supports this. Moreover, temporal signal characteristics correspond to the canonical HR shape and correlate with the stimulation time (Figure 3.1.1 A). The averaged task performance time across participants was 178 ± 16.78 s, and the averaged HR time obtained from first optode was 181.12 ± 18.92 sec and 182.5 ± 18.43 sec for the second. The mean task performance time and standard deviation were similar between optodes and within participants, but not identical.

3.1.3. Comparison with fNIR 400

The averaged fNIR 400 data showed the same HR delay in time for the left hemisphere as the manually designed system (Figure 3.1.1. B). The averaged task performance time across participants was 197 ± 13.90 s, and the averaged HR time obtained from optodes 11 and 12 was 199.12 ± 15.85 sec and 204.02 ± 15.34 sec for the optodes 5 and 6. Furthermore, using a paired t-test, it was found that HbO_2 and HbR concentrations were significantly higher in the left optodes 5 and 6 than in the right optodes 11 and 12, ($t(11) = 2.20$, $p = 0.49$).

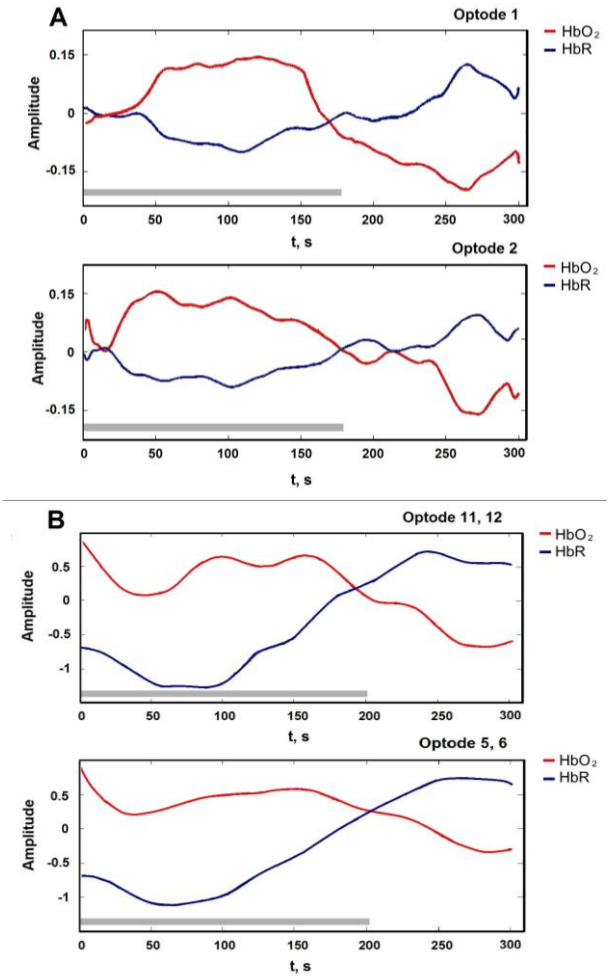

Figure 3.1.1. (A) The averaged fNIRS signals from the manually developed system grey bar - averaged per participants' task time (~178 s); averaged response time for optode 1 was 181.12 ± 18.92 sec and optode 2 was 182.5 ± 18.43 s. **(B)** The averaged fNIRS signals from fNIR 400: grey bar also indicates averaged per participants' task time (~197 s) and averaged hemodynamic response time for optodes 11 and 12 was 199.12 ± 15.85 sec and for optode 5 and 6 was 204.02 ± 15.34 s.

3.2. Investigation of sex and handedness effects on the WCST evoked the PFC HR

3.2.1. WCST

Consistent with previously reported findings, no statistically significant sex or handedness bias on WCST performance was observe (Eling et al., 2008; Nyhus & Barceló, 2009). The mean number of perseveration errors was 14.45 ± 6.50 (99 % confidence interval = 12.72, 16.18), and the mean correct sorting rate was 82.08 ± 7.78% (99 % confidence interval = 80.02, 84.14). No significant differences were found comparing by sex or handedness. The average duration of the WCST for all subjects was 268.74 ± 60.88 sec (99 % confidence interval = 252.87, 284.61). No statistically significant differences in the duration of task performance regarding sex or handedness were found (Table 3.2.1).

Table 3.2.1 The behavioural results of the WCST (mean ± SD).

Group	*N*	Perseveration errors, n	Correct sorting, %	Duration, sec
All	70	14.45 ± 6.50	82.08 ± 7.79	268.74 ± 60.88
Females	35	14.37 ± 6.00	81.50 ± 8.07	268.82 ± 42.82
Males	35	14.55 ± 7.09	82.74 ± 7.48	279.36 ± 48.36
Right-handed	40	14.30 ± 6.56	83.08 ± 6.88	284.01 ± 40.01
Left-handed	30	14.53 ± 6.45	82.49 ± 6.51	271.39 ± 67.39
Females Right-handed	20	14.47 ± 5.95	82.56 ± 6.23	264.66 ± 54.66
Females Left-handed	15	14.09 ± 7.38	83.72 ± 7.68	268.56 ± 58.56
Males Right-handed	20	14.70 ± 6.68	80.16 ± 9.37	280.25 ± 48.25
Males Left-handed	15	14.35 ± 6.39	82.49 ± 6.51	274.38 ± 54.38

3.2.2. fNIRS data

3.2.2.1. Between-dataset comparison by blocks

The results of the two-way RM-MANOVA test revealed that the main effect of sex was significant for pre-processed dataset ($F(2, 65) = 3.552$, $p = 0.034$, $\eta^2 = 0.099$), but not for the normalised dataset ($F(2, 65) = 0.949$, $p = 0.392$, $\eta^2 = 0.028$). Effect of handedness was in the edge of significance for pre-processed ($F(2, 65) = 2.959$, $p = 0.059$, $\eta^2 = 0.083$) and significant for normalised dataset ($F(2, 65) = 3.391$, $p = 0.040$, $\eta^2 = 0.094$), showing some

evidence that the null hypothesis does not hold when normalisation is used. However, the main effect of sex and handedness interaction was not significant for the pre-processed dataset ($F(2, 65) = 2.266$, $p = 0.112$, $\eta^2 = 0.065$) and beyond significance for the normalised dataset as well ($F(2, 65) = 2.946$, $p = 0.060$, $\eta^2 = 0.083$).

The analysis also revealed a highly significant interaction between hemodynamic changes over time ('1st MD block', 'WCST' and '2nd MD block') and in relation to sex regarding the pre-processed dataset ($F(4, 63) = 5.182$, $p = 0.001$, $\eta^2 = 0.248$), and still a significant interaction ($F(4, 63) = 2.639$, $p = 0.042$, $\eta^2 = 0.144$) for the normalised dataset. As can be seen from Figure 3.2.1, an analysis of the simple main effects shows that HbO_2 and HbR parameters were significantly changing over time (blocks),($F(4, 63) = 5.631$, $p < 0.0001$, $\eta^2 = 0.401$). Changes in each of the four HR parameters compared by sex and handedness in Figure 3.2.1 are shown for both datasets as estimated marginal means.

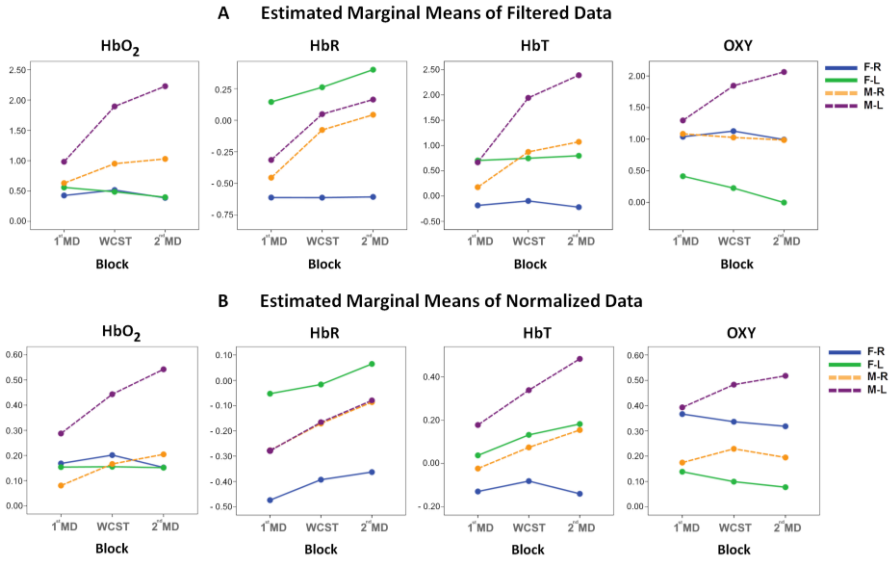

Figure 3.2.1. Plots of estimated marginal means for pre-processed **(A)** and normalised **(B)** data. The estimated marginal means between three experimental blocks and groups by sex and handedness: Modified Comparison (MC) block; Wisconsin Card Sorting Test (WCST); F– female; M– male; R– right-handers; L– left-handers.

3.2.2.2. Within-block comparison by sex and handedness

Similar results were obtained after comparing the pre-processed (Table 3.2.2), and normalised (Table 3.2.3) data of the main WCST block separately from the MC block. The results of the two-way RM-MANOVA test revealed that the main effect of sex was significant for pre-processed ($F (2, 65) = 3.521$, $p = 0.035$, Wilks' $\Lambda = 0.902$, $\eta^2 = 0.098$), but not significant for normalised block of WCST ($F (2, 65) = 1.093$, $p = 0.341$, Wilks' $\Lambda = 0.967$, $\eta^2 = 0.033$). Almost analogical effects were obtained on handedness: it was marginally nearly significant both for pre-processed ($F (2, 65) = 2.977$, $p = 0.058$, Wilks' $\Lambda = 0.916$, $\eta^2 = 0.084$) and normalised blocks of WCST ($F (2, 65) = 2.926$, $p = 0.061$, Wilk's $\Lambda = 0.917$, $\eta^2 = 0.083$). Meanwhile, the interaction between sex and handedness was not significant for pre-processed ($F (2, 65) = 2.271$, $p = 0.111$, Wilks' $\Lambda = 0.935$, $\eta^2 = 0.065$) and normalised blocks of WCST ($F (2, 65) = 2.749$, $p = 0.071$, Wilks' $\Lambda = 0.922$, $\eta^2 = 0.078$).

Some interactions regarding hemodynamic parameters were observed for the pre-processed dataset of the WCST block. The strongest statistically significant univariate effect was obtained considering sex and the HbO_2 measure ($F (1, 66) = 7.146$, $p = 0.009$, $\eta^2 = 0.098$). A moderately significant effect of handedness was obtained on HbR (please see Table 3.2.2 for details). HR derivatives, such as HbT and OXY, were impacted differently: sex as a ($p = 0.043$) less significant factor than handedness ($p = 0.022$) on the total haemoglobin; while oxygenation (difference between neurovascular supply and demand) was significantly affected by sex ($p = 0.042$), and more interestingly, it showed a sex by handedness interaction, ($F (1, 66) = 4.564$, $p = 0.036$, $\eta^2 = 0.065$). The statistical summary of between-subject effects can be found in Table 3.2.2. Moreover, the mean response for each factor, adjusted for all variables in the models, is shown in Figure 3.2.2 as an estimated marginal means. However, statistically less significant univariate effects were obtained on the normalised block of WCST: a significant effect of handedness on HbT ($F (1, 66) = 6,729$, $p = 0.012$, $\eta^2 = 0.093$), and previously obtained interactions of sex*handedness*OXY ($F (1, 66) = 6,175$, $p = 0.016$, $\eta^2 = 0.086$) were found (please see Table 3.2.3 and Figure 3.2.3 for details).

3.2.2.3. The significant between-group differences

An independent-sample t-test was conducted for the further investigation of differences between the means of independent groups of subjects

(female/male, n = 35/35; right-handed/left-handed, n = 40/30) for each HR parameter. Significantly different groups of subjects are additionally marked with asterisks and dotted lines in Figure 3.2.4.

Comparing the hemodynamic parameters, two-sample t-tests revealed that the HbO_2 concentrations of left-handed males (1.73 ± 0.29) differed significantly from right-handed (0.51 ± 0.27) and left-handed (0.48 ± 0.25) females, ($t(33) = -3.039$, $p < 0.05$) and ($t(28) = -3.230$, $p < 0.05$) accordingly. In addition, males tended to have higher overall measured HbO_2 concentrations than females (Figure 3.2.4). Further, there were statistically significant HbR concentration differences within females, comparing right-handers (-0.61 ± 0.17) and left-handers (0.26 ± 0.24), ($t(33) = 3.045$, $p < 0.05$). Examining the differences in the total haemoglobin increase between the groups of subjects, right-handed females (-0.10 ± 0.24) and left-handed males (1.63 ± 0.42) were significantly different ($t(33) = 3.807$, $p < 0.05$), whereas left-handed females and right-handed males were very similar (see Figure 3.2.3, where the y-axis values for the corresponding groups were at the same level). For the OXY parameter, a statistically significant concentration difference was found for left-handed females (0.22 ± 0.33) and males (1.82 ± 0.42), ($t(28) = -2.994$, $p < 0.05$), confirming the previously identified interaction of sex and handedness in the two-way RM-MANOVA test ($F(1, 66) = 4.564$, $p = 0.036$, $\eta^2 = 0.065$). As mentioned before, oxygenation is the difference between HbO_2 and HbR concentrations, or how much cerebrovascular overcompensation (the difference between the supply and demand) was present. Right-handed females and males were equivalent (see Figure 3.2.2, where the OXY plot y-axis values for right-handed groups match), and left-handed females and males were significantly different in their overall oxygen consumption during the WCST.

Table 3.2.2 Significant effects of sex and handedness on different hemodynamic parameters (N = 70, α = 0.05) from two-way RM-MANOVA tests (note: HbO_2, HbR and HbT, OXY pairs were examined separately) for a pre-processed block of the WCST. Significant values are bold. Only one statistically significant interaction of sex and handedness was obtained for oxygenation (OXY), (F (1, 66) = 4.564, p = 0.036, η^2 = 0.065). For more details on experimental design, see the workflow of fNIRS data preparation and statistical analysis (Figure 2.2.3; Materials and methods).

	Sex	**Handedness**
HbO_2	F (1, 66) = 7.146, p = 0.009, η^2 = 0.098	F (1, 66) = 1.942, p = 0.168, η^2 = 0.029
HbR	F (1, 66) = 0.003, p = 0.958, η^2 = 0.000	F (1, 66) = 4.338, p = 0.041, η^2 = 0.062
HbT	F (1, 66) = 4.244, p = 0.043, η^2 = 0.060	F (1, 66) = 5.519, p = 0.022, η^2 = 0.077
OXY	F (1, 66) = 4.322, p = 0.042, η^2 = 0.061	F (1, 66) = 0.006, p = 0.807, η^2 = 0.001

Figure 3.2.2. Estimated marginal means of the HR parameters regarding sex and handedness obtained on the pre-processed block of the WCST. Box and the star indicate significant differences between groups (p< 0.05). Results are from 70 subjects' two-way RM-MANOVA tests for the pre-processed block of the WCST.

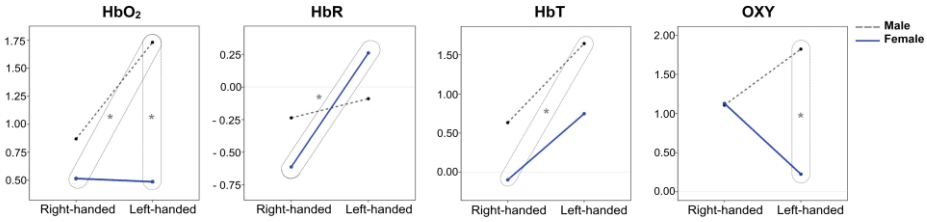

Table 3.2.3 Significant effects of sex and handedness on different hemodynamic parameters (N = 70, α = 0.05) from two-way RM-MANOVA tests (note: HbO_2, HbR and HbT, OXY pairs were examined separately) for a normalised block of the WCST. Significant values are bold. Only one statistically significant interaction of sex and handedness was obtained for oxygenation (OXY), (F (1, 66) = 6.175, p = 0.016, η^2 = 0.086). For more

details on the experimental design, see the workflow of the fNIRS data preparation and statistical analysis (Figure 2.2.3; Materials and methods).

	Sex	Handedness
HbO_2	F (1, 66) = 1.889, p = 0.174, η^2 = 0.028	F (1, 66) = 1.576, p = 0.214, η^2 = 0.023
HbR	F (1, 66) = 0.129, p = 0.721, η^2 = 0.002	F (1, 66) = 3.512, p = 0.065, η^2 = 0.051
HbT	F (1, 66) = 3.867, p = 0.053, η^2 = 0.055	F (1, 66) = 6.729, p = 0.012, η^2 = 0.093
OXY	F (1, 66) = 1.962, p = 0.166, η^2 = 0.029	F (1, 66) = 0.007, p = 0.932, η^2 = 0.000

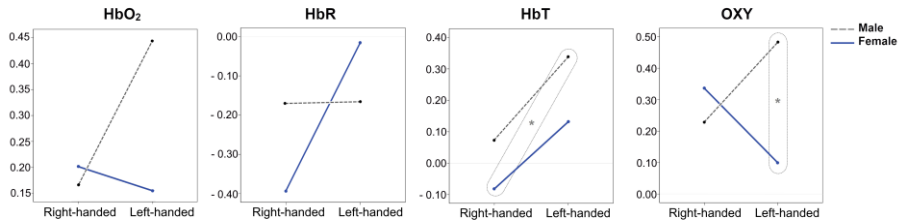

Figure 3.2.3. Estimated marginal means of the HR parameters regarding sex and handedness obtained on a normalised block of the WCST. The box and the star indicate significant differences between groups ($p < 0.05$). Results are from 70 subjects' two-way RM-MANOVA tests for the normalised block of the WCST.

3.2.2.4. Significant sex effects on the HbO_2 concentration

The strongest statistically significant simple main effect was obtained considering sex and HbO_2 concentrations (p = 0.009; see Table 3.2.2). Males, in general, tended to have higher overall measured HbO_2 concentrations than females, and left-handed males differed significantly from both groups of females (Figure 3.2.4). Independent two-sample t-tests were used *to* demonstrate the spatial differences in HbO_2 concentrations between groups of subjects: the error plots in Figure 3.2.4 A show the distribution of the mean (± SD) concentrations of HbO_2 in 16 optodes (left), and the mean (± SD) across optodes per group (right). The variability of means between groups is apparent and consistent (Figure 3.2.4 A from left to right: right-handed females, left-handed females, right-handed males, and left-handed males; also see

Table 2.2.1 for details). The statistical activation maps (t-values, $p < 0.05$) in Figure 3.2.4 B show where HbO_2 was significantly higher for left-handed males than right-handed or left-handed females. The substantial differences between these three subject groups for HbO_2 are obtained in the right superior frontal gyrus (9–12th optodes) and the left middle frontal gyrus (2–4th optodes).

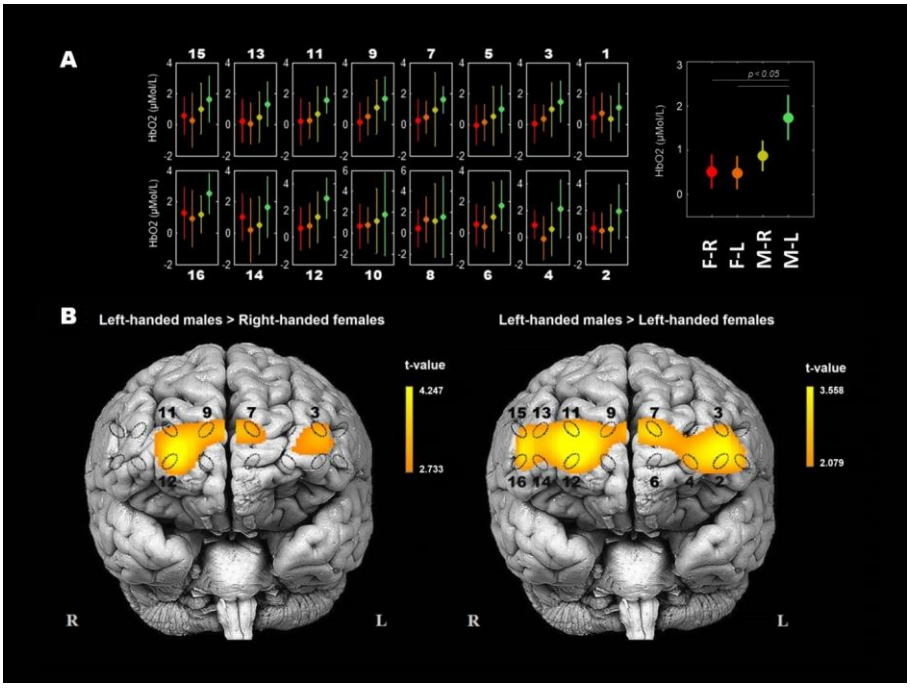

Figure 3.2.4. (A) Error plot of the HbO_2 mean (± SD) concentrations of HbO_2 between groups in each of 16 optodes (left), and the mean (± SD) concentrations of HbO_2 across optodes per group (right). Subjects groups are females right-handed, n = 20, F-R; females left-handed, n = 15, F-L; males right-handed, n = 20, M-R; males left-handed, n = 15, M-L). **(B)** Statistical activation maps (t-values, $p < 0.05$) display where the obtained HbO_2 concentrations were significantly higher for left-handed males than right-handed females ($t(33) = 3.039$, $p < 0.05$) and left-handed females ($t(28) = 3.230$, $p < 0.05$). Significant optodes are outlined. Statistical values were spatially visualised with the BSpline18 interpolations method. The measured areas are marked with ellipses.

3.2.2.5. The interaction between sex and handedness regarding oxygenation

The oxygenation may be interpreted as a measure of how much of cerebrovascular overcompensation, or the difference between supply and demand, is present at the particular brain area. At this point, a statistically significant interaction between sex and handedness on OXY was found (see Tables 3.2.2 and 3.2.3). The measure of oxygenation is not often used, as it does not directly correspond to current biophysical models of fNIRS or fMRI and is considered more as an additional measure. However, its physiological interpretation may give some insights into cerebrovascular regulation: the cerebrovascular reactivity (the active vasodilatation of neurovasculature to increase oxygen supply) and relative metabolic rates of oxygen consumption (corresponding to the level of brain tissue rather than cellular metabolism). Considering previous findings, right-handed females and males are very similar (Figures 3.2.2 and 3.2.3), and left-handed females and males are significantly different ($t(28) = -2.994$, $p < 0.05$; pre-processed dataset). The significant interaction can be subsequently explained by the significant effects of sex and handedness on HbO_2 and HbR, as the oxygenation differs between these two parameters.

Nevertheless, 2D contour plots were created to demonstrate the spatial and temporal distribution of the OXY during the WCST (Figure 3.2.5). Figure 3.2.5 shows the qualitative and quantitative oxygenation distribution for all four subjects' groups during the performance on the WCST. Because the duration of the WCST performance was individualised (see 3.1), participant's individual data were normalised in time and amplitude before the group average. Normalisation in time was made from the start of the WCST (0), and to the end of the WCST (1) individually. The amplitude was scaled by its maximum value as introduced before and corresponds to the normalised dataset.

As a result, the temporal dynamics of OXY could be defined as quite steady during the WCST performance in all groups, except for left-handed men, who reach their peak activity in the last quarter of the experiment. Frontal brain views in the lower part of Figure 3.2.5 show the localisation of the within-group means signal intensities, which is quite distinctive between groups. To sum up, oxygenation differences between subjects regarding their sex and handedness demonstrate the cerebrovascular reactivity and relative metabolic rates of oxygen consumption creating significantly different patterns.

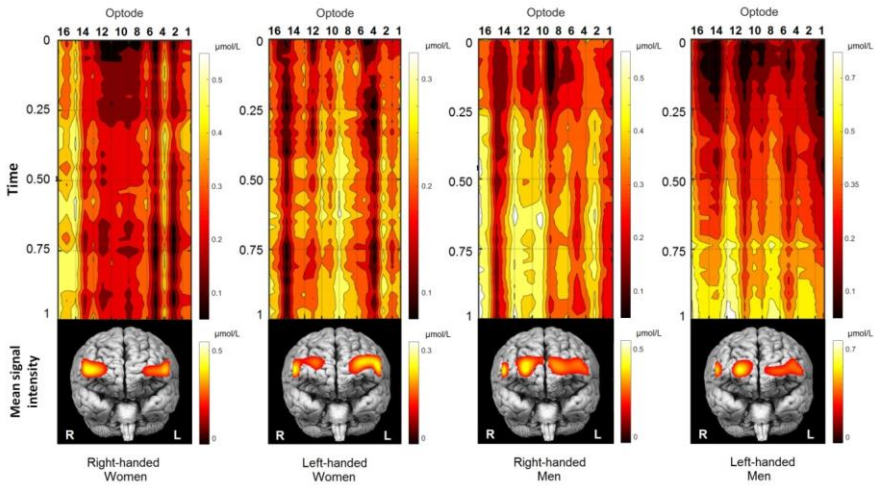

Figure 3.2.5. Visualisation of time-series OXY, together with mean spatial signal distribution on the frontal brain view. All 70 subjects were divided into four groups by sex and handedness. Time-series of OXY concentrations were normalised by the maximum value. The mean signal is shown below. Recordings were also normalised in time (from the start of WCST being 0, and to the end of WCST being 1) individually and later averaged per group. Note that the time series presentation does not correspond directly to the frontal brain view distribution below due to double line probe geometry. Values were spatially visualised with BSpline18 interpolations method.

3.2.2.6. Within-block comparison by hemisphere and the LI

The results of the two-way RM-MANOVA test regarding hemispheric activity revealed significant HbR differences between hemispheres in the pre-processed ($F(1, 66) = 6.479$, $p = 0.013$, $\eta^2 = 0.089$), and highly significant differences between hemispheres in the normalised block of the WCST ($F(1, 66) = 12.141$, $p = 0.001$, $\eta^2 = 0.155$). However, no effects of sex or handedness were obtained on HbO_2 or HbR. Moreover, HbO_2 demonstrated only a slight tendency toward statistical significance in the pre-processed ($F(1, 66) = 3.234$, $p = 0.077$, $\eta^2 = 0.047$) and normalised ($F(1, 66) = 2.614$, $p = 0.111$, $\eta^2 = 0.038$) datasets. Similarly, there were significant HbT differences between hemispheres in the pre-processed ($F(1, 66) = 8.774$, $p = 0.004$, $\eta^2 = 0.117$) and normalised ($F(1, 66) = 4.602$, $p = 0.036$, $\eta^2 = 0.065$) datasets, but no significance regarding OXY in the pre-processed ($F(1, 66) = 0.072$, $p = 0.789$,

$\eta^2 = 0.001$) and normalised ($F(1, 66) = 0.011$, $p = 0.915$, $\eta^2 = 0.000$) datasets. No effects of sex or handedness were obtained on hemispheric hemodynamic differences.

The LI also addressed no clear hemispheric dominance, and the results show no consistent pattern across subjects. In most cases, a right-sided dominance was slightly more favoured for the HR parameters (LI was negative from 46 to 54% for all subjects), except the OXY concentration where a slight favour of left-sided hemisphere dominance was present (LI was positive for 47% of all subjects, and negative and bilateral values were almost equal, for 29% and 24% of all subjects, respectively),(Figure 3.2.6). However, no significant relationships between LI and sex or handedness individually or together were found.

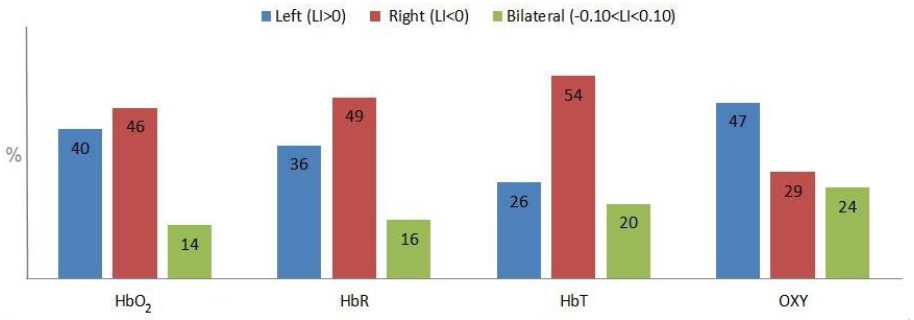

Figure 3.2.6. The percentage of the LI within HR measures; LI = (L – R)/(L + R), where L and R indicate the sum of the mean values of the concentrations on the left and right hemispheres. No consistent or significant patterns across subjects (n = 70) were obtained independently or concerning biological features. LIs are given for the normalised dataset as the pre-dominant handedness, and language lateralisation could potentially cause the shift. However, no significant effects were found in the pre-processed dataset as well.

4. DISCUSSION

The results of the first research project for developing a CW-fNIRS system for human frontal lobe studies demonstrated that manually designed CW-fNIRS system could be used for the frontal lobe studies in human adults. Furthermore, the results indicate small, but measurable differences in hemispheric activation that was particularly obtained on the fNIR 400 device. This can be explained by the higher number of optodes used for measurements. However, from a previous literature review, it was not clear whether the WCST demonstrates clear hemispheric dominance or a specific activation pattern among prefrontal cortex regarding other factors. Thus, regarding the experimental findings, a follow-up literature review has been done to identify whether the obtained consistent differences in activity between frontal lobes and across devices are evident in previous literature. It was found that the WCST show very inconsistent results between and within patients and even healthy controls. Moreover, few studies have been done using fNIRS. Thus, after the first research project the decision of the author has been made to initiate extensive research on the prefrontal activation caused by the WCST using fNIR 400. The second research project particularly investigated sex and handedness effects on the WCST evoked a PFC HR due to the variability obtained in group composition in previous studies. As the second research project accounts for most of the findings in the dissertation, the discussion and conclusions are built on it.

4.1. Sex-related HR differences among subjects

Analyses of the second research project revealed significant sex-related HR differences among subjects in HbO_2 concentrations – males demonstrate higher overall measured HbO_2, concentrations than females (Figure 3.2.4 A). In contrast, it has been shown that brain perfusion or cerebral blood flow (CBF) is notably increased in the PFC of females (Amen et al., 2017). However, according to the current study results, males demonstrate higher overall measured HbO_2 concentrations than females. At the same time, several other fNIRS studies on the prefrontal cortex also found the amplitudes of HbO_2 being significantly higher in males than females during a working memory task (Li, Luo, & Gong, 2010) and mental arithmetic (Yang et al., 2009). The same results were obtained for a resting state (Jaušovec & Jaušovec, 2010). These findings do not necessarily contradict each other, as

perfusion is defined by CBF, where HbO_2 is related to CBF and cerebral blood volume (CBV). CBF is maintained by a decrease in the resistance of the distal vasculature, where as a result, both CBV and the CBV/CBF ratio would increase, while the extraction of oxygen from the circulating blood may remain the same (Powers, 1991; Toga, 2015). In other words, cerebrovascular regulation is a dynamic process, and different neuroimaging methods are based on different biophysical models of the same phenomenon and thus provide various data.

Unfortunately, only a few WCST studies have been done with fNIRS (Fallgatter & Strik, 1998; Hashimoto et al., 2007; Sumitani et al., 2006; Venclove et al., 2015). One was a pilot study for the currently presented first research project (Venclove et al., 2015). It raised two important questions: (a) regarding whether contradictions between neuroimaging and behaviour exist within other cognitive tests, particularly those who usually do not show sex bias; (b) whether similar or contrasting findings were observed in resting state functional studies. The first question can be illustrated with the same fNIRS studies, where sex-related differences in HRs have been reported during verbal working memory (Li et al., 2010) and mental arithmetic (Yang et al., 2009) tasks. Both studies found that the amplitudes of HbO_2 were significantly higher in males than females and to some extent, this was interpreted as evidence of different information-processing patterns, although sex-related behavioural differences were not found. Both tasks are presumably considered to demonstrate sex-related differences elsewhere (Lenroot & Giedd, 2010; Ruigrok et al., 2014). However, the authors do not provide a clear explanation and supporting evidence as to why the lack of correspondence between functional neuroimaging and behavioural results is considered evidence of different cognition. As discussed, the astroglial network (Giaume et al., 2010; Scemes & Spray, 2003) and other non-neural origin cells (Hamel, 2006; Sweeney et al., 2016) have been recently shown to have a considerable impact on normal neurovascular dynamics. Moreover, their conclusions are highly debatable, as the findings obtained with fNIRS and fMRI studies demonstrate nonlinear properties of the HR during task performance (Fabiani et al., 2014; Huneau et al., 2015; Wager, Vazquez, Hernandez, & Noll, 2005). Regarding the second question, only one study solely based on the fNIRS-based resting state was found exploring sex-related differences in prefrontal cortex activity in a total of 40 healthy right-handed subjects (Chuang & Sun, 2014). This fNIRS resting-state functional connectivity study proposed that inferior PFC is significantly different between male and female groups with both time-

series and spectrum analyses (Chuang & Sun, 2014). However, no overall haemoglobin concentrations comparison were provided. Another resting state study of 300 healthy right-handed subjects was assessed by fNIRS and EEG in a whole brain approach (Jaušovec & Jaušovec, 2010). The researchers proposed that males display a higher level of HbO_2 than females (Jaušovec & Jaušovec, 2010). The authors also indicated that males and females significantly differ in the default mode of brain activity considering both modalities (Jaušovec & Jaušovec, 2010). In contrast, no comparable resting state studies in healthy subjects regarding sex differences were found with widely used fMRI, as the overall BOLD signal intensities are not usually compared, and more sophisticated methods are proposed instead (Biswal, 2015; Lee, Smyser, & Shimony, 2013).

At this point, sex-related effects on functional neuroimaging should be separated into those due to sexual dimorphism and those thought to appear due to sex hormones and sex hormone-modulated neurochemistry. There are relatively accepted overall sex differences in brain volume and morphology (Allen et al., 2002; Lenroot & Giedd, 2010; Ruigrok et al., 2014), the grey/white matter ratio (Gur et al., 1999) and structural asymmetry (Kong et al., 2018). However, possible differences in some aspects of the local capillary bed are still poorly addressed (Mishra et al., 2016; Walsh, 2016). It is unknown whether the density of local capillary network is primarily consistent within the brain, or there are some asymmetries across the cerebrovasculature as well, as it is evident with previously discussed brain features. If this structural sexual dimorphism regarding cerebrovasculature would be the case, then, for example, a scaling or adjustments by a local capillary bed density in functional data could be implemented for an accurate comparison, as is currently done with structural brain images. Subsequently, it is also relatively unknown how different hormonal environments affect the dynamic properties of cerebrovasculature, not only between sexes,- because the additional analysis regarding menstrual cycle within females by their menstrual cycle gave no particular results. The absence of differences between menstrual phases supports the idea that not all discrepancies can be explained solely by female hormones' fluctuations. On the other hand, a menstrual phase was identified only through the questionnaire and not by a more accurate blood or saliva test. Moreover, no equivalent evaluation of hormonal composition for men was done. Thus, it is still plausible that some functional brain maps of the WCST in the current study are sensitive to neurophysiological heterogeneity, which is not apparent or necessarily related to the behaviour.

However, it cannot be excluded that visible heterogeneity in neuroimaging results is due to cognitive sub-processes that cannot be detected on a behavioural level. However, despite its importance, the understanding of NVC is still incomplete, and the implementation of more recent cellular level findings and computational modelling on existing HR paradigms is highly favourable to solve this question (Hillman, 2014; Sweeney, Walker-Samuel, & Shipley, 2018). In brief, the current study has demonstrated that sex has a systematic confounding effect on HRs, and the data normalisation procedure eliminates most of the sex-related differences during the WCST and significantly impacts the optical spectroscopy research results (see Tables 3.2.2 and 3.2.3).

4.2. Handedness-related HR differences among subjects

Meanwhile, the effects of handedness are more complex than ambiguous: for the chosen alpha level, it was not statistically significant at the multivariate level, but the moderately significant effect of handedness was obtained alone, or in relation to sex (see Tables 3.2.2 and 3.2.3; Figures 3.2.2, 3.2.3 and 3.2.4; Results). This study demonstrates that normalisation does not eliminate and rather strengthens the significant effects of handedness by eliminating sex effects. As previously, no effects were apparent on the behavioural level of the WCST performance as well (see Table 3.2.1; Results). This leads to the hypothesis that more than one brain circuit can give similar behavioural outputs. Indeed, a recent investigation of the atypical lateralisation of language in healthy subjects suggests that handedness and language lateralisation are related only indirectly, but left-handedness increases the likelihood of bilateral or right-hemispheric language specialisation, which in turn alters brain connectivity and even leads to the changes of the global signal in a resting state (Bidula et al., 2017; Chuang & Sun, 2014). Moreover, the natural variation of language lateralisation is considered a part of a natural continuum of hemispheric specialisations, and in some cases could potentially better explain apparent alterations in the neuropsychological effects of handedness than a conventional explanation based on the opposite causality. In brief, moderate handedness effects on HR measures might be explained by an indirect connection with normal variability in language lateralisation, which affects brain networks on the global scale. And this is apparent with and without the systematic confounding effect of sex. Unfortunately, there is no easily accessible way to check for language lateralisation, except the already

defined relationship with handedness (Hervé et al., 2013; Mellet et al., 2014; Prichard, Propper, & Christman, 2013).

4.3. Hemispheric dominance and laterality

Continuing with hemispheric dominance, the investigation of hemispheric dominance and laterality did not show promising results. The current research found contradicting results: even though RM-MANOVA suggests that the left hemisphere is favoured across participants regarding HbR and HbT, the individual evaluation by LI suggests a slightly favouring of the right hemisphere; and the complete opposite can be obtained regarding OXY; no clear lateralisation was obtained by any method. This is consistent with other functional neuroimaging studies where it has been demonstrated that the WCST fails to distinguish laterality of the brain damage and demonstrates no consistency across different neuroimaging studies (Nyhus & Barceló, 2009). This could be explained by the fact that the retrieval of episodic memories critically depends on effective inter-hemispheric interaction (Burgess, Simons, Dumontheil, & Gilbert, 2004; Prichard et al., 2013).

4.4. Limitations and future directions

Limitations

Despite the essential findings that have emerged in this study, some equally important criticism needs to be addressed for future research.

Regarding the first research project, the relatively small sample size should be acknowledged. Since the purpose of the use of the WCST was to test the sensitivity of the device, it can be justified by a methodologically distinct approach and pilot study. Nevertheless, the comparison of a custom-designed fNIRS system with a currently used commercial fNIRS device fNIR 400 is not straightforward due to differences in baseline recording. This was due to technological and signal processing differences, as fNIRS 400 used a standard 10 second time frame for it. However, this methodological issue could be thoroughly addressed in similar future comparisons by recording and processing a separate baseline with fNIR 400 to make it more equal with a custom-designed device.

Further, regarding both projects, the possible contamination of fNIRS data that could alter some of the results cannot be entirely discarded. The fNIRS signal contamination from extra-cerebral tissues on

overall hemodynamic measurements is hard to overcome, due to a path of light being unavoidably heterogeneous (Tachtsidis & Scholkmann, 2016). Several steps minimised the possible impact. First, all subjects in this study were Caucasians, and acceptable signal levels for measurements were reached by individually adjusting light intensities before the experiment. Second, a tight fit of the probe to the head to squeeze out the scalp blood was ensured. However, without explicit control extra-cerebral contamination cannot be entirely discarded, as most of the fNIRS signal is thought to come from the brain surface where the cortical vasculature is very dense (Mishra, 2017; Reina-De La Torre, Rodriguez-Baeza, & Sahuquillo-Barris, 1998). At this point, apparent differences between men and women cannot be explained solely by scalp-origin physiological confounds without empirical proof. Moreover, even if this would be the case, most of the commercially available CW-fNIRS devices still do not have implemented solutions (Tachtsidis & Scholkmann, 2016). This illustrates the need for the fNIRS community to define some clear and consistent data-processing guidelines to ensure proper and widely accepted inference strategies.

As such, the implementation of a data normalisation approach clearly showed that the original data distribution is a combination of at least two overlapping distributions that can be preliminarily defined by sex. Thus, during the statistical analysis of fNIRS data, the sex as a factor should be accounted for by including it as cofounding factor or performing analysis with already normalised data. Moreover, due to sex-related odds ratios for left-handers, normalisation in the presence of sex effects might be suggested as a suitable method to capture less prominent handedness-related effects during the WCST performance. Moreover, to reduce the amount of possible interactions in data processing itself, the current study intentionally avoids overuse of complicated data cleaning, processing and analysis procedures to assure that the final results are not due to any interferences of these choices. Thus, more sophisticated approaches could be addressed in future studies.

Another limitation that should be kept in mind regarding the second study is that only the overall effect of the WCST on the HR was investigated. Due to the nature of the WCST and a low sampling rate of fNIRS 400, it was not possible to observe the HR during category shifting. This potentially reduces our understanding of sex and handedness-related interactions on the WCST. Moreover, a whole brain approach could give better insight, as frontal and non-frontal cortical activations during the WCST could be subsequently addressed (Nyhus & Barceló, 2009).

Another concern, rather distinct from the main dissertation goal, but still important as a whole to address, is that no spatial normalisation is currently implemented (see Section 1.2.3; Literature review) to discard natural sex differences in brain volume and morphology, which also may potentially impact the current results.

Future directions

In addition to everything discussed above, the dissertation briefly covers two at first glance distinct, but likely interconnected problems where the common denominator is the functional neuroimaging. First, the shared knowledge of the complex neurophysiological reasoning regarding sex and handedness is still quite often used in favour of a researcher's goal to strengthen a conclusion by *a priori* or *a posteriori* excluding women, or left-handers after observing some discrepancies in their results (Hill et al., 2014; Hirnstein et al., 2014; Miller & Halpern, 2014; Willems et al., 2014). From this perspective, cognitive and behavioural studies do not always investigate further whether obtained inconsistencies or contradictions between brain maps and behaviour are due to underlying different cognitive processes, or to possible variations in neurophysiologic signal transduction. Second, functional neuroimaging techniques are based on NVC and do not directly measure neuronal activity. Over the last decades, the understanding of NVC initiation and its overall role dramatically changed from the previous "neuron-centric" supportive perspective to the recognition that glial cells are equal contributors in the healthy central nervous system and pathogenesis of many brain disorders (Liddelow & Barres, 2017). Nevertheless, our understanding of NVC in humans despite its importance for accurately translating and understanding functional neuroimaging results is still incomplete (Hillman, 2014; Phillips et al., 2016). Altogether, this means that ultimately it is recommended that any observation about differences in cognitive strategies by sex and handedness from the studies using functional neuroimaging should be supplemented with electrophysiological evidence, especially then no clear behavioural difference is obtained.

CONCLUSIONS

1. During the WCST, males demonstrate higher concentration levels of HbO_2 in the prefrontal cortex than females, and particularly left-handed males;

2. Left-handed males show statistically significant higher concentrations of HbO_2 during the WCST than both groups of females, but not right-handed males: the substantial differences between left-handed males and females are in the right superior frontal gyrus (9–12th optodes) and the left middle frontal gyrus (2–4th optodes);

3. A strong statistically significant interaction between sex and handedness was obtained regarding OXY: left-handed males show a significantly higher concentration than left-handed females in the pre-processed and normalised blocks of the WCST, while values of right-handed males and females are comparable;

4. The data normalisation procedure affects the statistical outcomes of sex and handedness effect: sex-related effects were mostly cancelled out, except previously identified strong relationship with handedness obtained regarding OXY;

5. The optical neuroimaging results do not correspond with behavioural results during the WCST in healthy subjects: no sex or handedness effects were obtained on a behavioural level;

6. There is no clear functional asymmetry in the prefrontal cortex during the WCST when measured with fNIRS.

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SUPPLEMENTARY MATERIAL

Supp.1. The Flinders Handedness Survey.

The original English version: M. E. R. Nicholls et al., "The Flinders Handedness survey (FLANDERS): a brief measure of skilled hand preference", *Cortex*. **49**(10), 2914–2926, Elsevier Ltd (2013), [doi:10.1016/j.cortex.2013.02.002].

The first use of Lithuanian version: Genyte, V., Griskova-Bulanova, I. "Auditory Steady-State Response: The Impact of Handedness and Sex", proceedings of the international conference *Virtual instruments in biomedicine*, 2015, p. 54–58. *Reused with the permission.*

Pavardė:……Vardas:……...……...…
Gimimo data:…… …… Lytis (vyras/moteris)……

Jums bus pateikta dešimt klausimų apie tai, kurią ranką Jūs naudojate skirtingose situacijose. Prašome varnele pažymėti vieną atsakymo langelį, labiausiai nusakantį, ar tam tikrai užduočiai atlikti naudojate dešinę, kairę ar abi rankas vienodai. Atkreipkite dėmesį, kad atsakymą „abiem" pasirinkite tik tuo atveju, kai nei viena iš rankų nėra pranašesnė už kitą. Prašome atsakyti į visus pateiktus klausimus, net jei su kažkokia užduotimi nesate susidūręs - tiesiog pasistenkite įsivaizduoti kaip galėtumėte ją atlikti, ir pažymėkite atsakymo variantą.

		Kaire	Abiem	Dešine
1.	Kuria ranka Jūs rašote?			
2.	Kuria ranka naudojate šaukštą, kai valgote?			
3.	Kurioje rankoje laikote dantų šepetėlį, kai valotės dantis?			
4.	Kurioje rankoje laikote degtuką, norėdami jį uždegti?			
5.	Kurioje rankoje laikote trintuką, kai trinate pieštuku padarytas klaidas?			
6.	Kurioje rankoje laikote adatą, kai siūnate?			
7.	Kai tepate sviestą ant riekės duonos, kurioje rankoje yra peilis?			
8.	Kuria ranka laikote plaktuką?			
9.	Kurioje rankoje laikote skustuką, kai lupate obuolį?			
10.	Kuria ranka piešiate?			

Rankiškumo balas *(prašome nepildyti):*	

LIST OF PUBLICATIONS

PUBLICATIONS RESULTING THE DISSERTATION**:**

Journal papers

S. Cinciute, "Translating the hemodynamic response: why focused interdisciplinary integration should matter for the future of functional neuroimaging", in PeerJ [Internet], 2019, vol: 7:e6621. https://doi.org/10.7717/peerj.6621

S. Cinciute, A. Daktariunas, and O. Ruksenas, "Hemodynamic effects of sex and handedness on the Wisconsin Card Sorting Test: the contradiction between neuroimaging and behavioural results", in PeerJ [Internet], 2018, vol: 6:e5890. https://doi.org/10.7717/peerj.5890

S. Cinciute, A. Daktariunas, and O. Ruksenas, "Functional Near-Infrared Spectroscopy: A Continuous Wave Type Based System for Human Frontal Lobe Studies", in EXCLI journal [Internet], 2015, vol.14, October, pp. 1145-1152. ISSN 1611-2156. https://dx.doi.org/10.17179%2Fexcli2015-614

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Conference Proceedings

R. Razinskaite, **S. Cinciute**, "Menstrual Cycle Influence on Functional Hyperemia in Prefrontal Cortex", in 9th International Scientific Conference of the Association of Lithuanian Neurosciences, Kaunas, 1 December 2017.

S. Cinciute, A. Daktariunas, and O. Ruksenas, "Sex Differences In Frontal Lobe Hemodynamic Response During Cognitive Task Performance", in *Biennial meeting of The Society for functional Near Infrared Spectroscopy*, Paris,13-16 October 2016.

S. Cinciute, A. Daktariunas, and O. Ruksenas, "Sex Differences In Frontal Lobe Hemodynamic Response During Cognitive Task Performance", in *Aspects in Neuroscience*, Warsaw, 25 November 2016.

OTHER PUBLICATIONS:

Conference Proceedings

I. Zelionkaite, A. Pleskaciauskas, **S. Cinciute**, "Absence of sex differences in microsaccadic eye movement parameters while perceiving illusory motion", in 9th IMPRS NeuroCom Summer School in Cognitive Neuroscience at the Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, 16-19 June 2019.

S. Cinciute, B. Draganski, "The subjectiveness of illusory motion perception: evidence from effective connectivity study", in 24th Annual Meeting of the Organization for Human Brain Mapping, Singapore, 17-21 June 2018.

V. Labanauskas A. Daktariunas, V. Valiulis, **S. Cinciute**, K. Dapsys, "Application of fNIRS for Diagnosing Major Depressive Disorder and Evaluating Treatment Effectiveness of Transcranial Magnetic Stimulation", in 9th International Scientific Conference of the Association of Lithuanian Neurosciences, Kaunas,1 December 2017.

I. Zelionkaite, **S. Cinciute**, A. Pleskaciauskas, "Microsaccades and Illusory Motion", in 7th International conference "Aspects in Neuroscience", Warsaw, 24-26 November 2017.

S. Cinciute, A. Ruef, L. Melie-Garcia , L. Jaugey, M. P. Strippoli, V. Rostant, G. Roccia, P. Marquet, J.M. Aubry, M. Preisig, F. Kherif, B. Draganski, "Applicability of quantitative MRI techniques for the assessment of abnormal microstructure properties of brain tissue in mood disorders", in International conference "NEUBIAS 2020", Lisbon, 15-17 February 2017.

ABOUT THE AUTHOR

Professional Experience

The author worked as a specialist at the Institute of Biosciences, Vilnius University from 2013 to 2019, resulting in the successful implementation of two functional neuroimaging research methods: functional near-infrared spectroscopy (fNIRS) and functional magnetic resonance imaging (fMRI). The author also gave short lectures at the university, conducted laboratory work, assisted in final exams, advised and supervised students' final theses:

Kazakov, I. (2019). Methods of segmentation of anatomical images of the human brain for early preoperative diagnosis of tumours: Master's thesis in Neurobiology. Vilnius: Vilnius University.

Razinskaite, R. (2019). The influence of spatial normalization on the assessment of cognitive functions in brain tumours: Bachelor's thesis in Biophysics. Vilnius: Vilnius University.

Zelionkaite, I. (2018). Influence of microsaccades on the perception of illusory motion: Master's thesis in Neurobiology. Vilnius: Vilnius University.

Education

Ph.D in Biophysics	*2015 – 2019*
M.Sc in Neurobiology	*2013 – 2015*
B.Sc in Biophysics	*2009 – 2013*

Vilnius University, Lithuania

Courses & Certificates

2018	*"Educational Courses of Organization for Human Brain Mapping 2018",* 1 day onsite courses, Singapore *"Stepping into the Future – a Multi-Disciplinary Approach to Brain-Machine Interaction",* 2 days onsite courses and symposium held in Vilnius, Lithuania
2017	*"The Second Training School of NEUBIAS for bioimage analysis",* 2 days onsite courses, IGC Gulbenkian Institute of Science, Oeiras, Portugal
2016	*"Educational Courses of Resting State and Brain Connectivity Conference 2016",* 2 days onsite courses, University of Vienna, Vienna, Austria *"Neuroimaging and fMRI Clinical and Research Applications",* 5 days onsite courses in Vilnius, Lithuania *"Educational Courses of Organization for Human Brain Mapping 2016",*1 day onsite courses in Geneva, Switzerland

	"Statistical parametric mapping", 6 days onsite courses, University of Lausanne, Lausanne, Switzerland
	"Basic analysis of fMRI data", 2 days onsite courses, University of Fribourg, Fribourg, Switzerland
2015	*"3rd Autumn School In Optical Imaging And Cerebral Oximetry"*,40 hours onsite courses, Amiens, France
	"Principles of fMRI", 4 week online courses, Johns Hopkins University, Baltimore, USA
	"Statistical Analysis of fMRI Data", 4 week online courses, Johns Hopkins University, Baltimore, USA

Organizations

since 2016	Organization for Human Brain Mapping (OHBM)
since 2016	Society for Functional Near Infrared Spectroscopy (SfNIRS)
since 2012	Federation of European Neuroscience Societies (FENS)
since 2012	Lithuanian Neuroscience Association (LNA)

Selected skills

- Biomedical physics and engineering
- Biochemistry and molecular biology
- Human anatomy and physiology
- Neurosciences
- Laboratory animal science
- Matlab
- Microsoft Office
- SPSS, Origin
- SPM12
- Windows, Linux

Languages:

- Lithuanian (Native)
- English (Proficient)
- German (Basic)
- Russian (Basic)

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