Imperial College London

Examining targeted brain stimulation to improve vigilant attention in right-hemispheric stroke

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Declaration of Originality

Dr Paresh Malhotra has supervised the preparation of this thesis. I also consulted Professor David Soto in regard to the signal detection theory analyses described in Chapter IV.

Screening and consent on stroke wards have been performed by myself and Dr Marianna Kapsetaki, in collaboration with Jenny Crow and Dr Meenakshi Nayar. A subset of older adults has been recruited with the assistance of Dr Korina Li.

Shuler Xu performed the electric field simulation in Sim4Life described in Chapter III.

Dr Ines Violante provided me with the methods for the functional imaging analyses described in Chapter VI. All fMRI analyses were performed adapting code originally written by Dr Violante. She created the mask encompassing the electrodes that was used to examine local connectivity.

Medical students Toby Sinclair and Ara Faraj assisted me with the collection of a subset of data presented in Chapter V and VI, as part of their lab placement. Another visiting student, Roberta Calvo, supported me with lesion mapping and helped me curate the stroke database. Lesion maps for two patients were delineated by Dr Marianna Kapsetaki as part of another study.

All other work contained in this thesis is entirely my own, and all sources of information derived from the work of others have been appropriately referenced. To the best of my knowledge, this thesis conforms to the rules and guidelines set out for PhD theses by Imperial College London.

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To Leonardo

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Statement of Publications

During my PhD, I published a chapter in a book series by Progress in Brain Research (not part of this thesis) (Olgiati et al., 2016).

Elements of Chapter I and Chapter III of this thesis have been published in a special issue on neglect by the Neuropsychological Rehabilitation journal (Olgiati & Malhotra, in press).

Elements of the stimulation montage that I designed (detailed in Chapter 3), together with my preliminary findings from the patient behavioural study (Chapter V) and my imaging study (Chapter VI), have been published in abstract form by the Brain Stimulation journal (Olgiati et al., 2019).

Manuscripts for the findings described in Chapter IV, V and VI are in preparation.

Olgiati, E., Russell, C., Soto, D., & Malhotra, P. (2016). Motivation and attention following hemispheric stroke. In B. Studer & S. Knecht (Eds.), *Progress in Brain Research* (Vol. 229, pp. 343-366): Elsevier.

Olgiati, E., & Malhotra, P. (2020). Using non-invasive transcranial Direct Current Stimulation for Neglect and Associated Attentional Deficits following Stroke. *Neuropsychol Rehabil*.

Olgiati, E., Violante, I.R., Li, L.M., Faraj, A., Sinclair, T., Crow, J., Wise, R., & Malhotra, P. (2019). Targeted brain stimulation to ameliorate vigilance in stroke: a combined tDCS-fMRI approach. *Brain Stimulation* 12(2):573.

Abstract

Neglect is a disabling neuropsychological syndrome frequently observed following right-hemispheric stroke. Affected individuals present with attentional deficits, ranging from a difficulty in orienting towards the contralesional space to a generalised difficulty with maintaining attention over time. Neglect may be persistent - particularly its non-lateralised component.

This thesis focused on investigating the efficacy of a potential treatment involving non-invasive targeted brain stimulation to improve vigilant attention. In a randomised double-blind sham-controlled crossover study, healthy individuals across the lifespan and stroke patients with attentional impairments received real and sham transcranial direct current stimulation (tDCS) whilst performing a vigilance task. A high-definition montage was used to constrain current delivery over the right dorsolateral prefrontal cortex, a key region of the vigilance network.

Results show that, at the group level, targeted tDCS improved target detection across all groups. By examining performance across temporal epochs, it was noted that tDCS did not impede worsening of performance with increasing time-on-task. The superiority of tDCS was however found throughout the task, outlasting stimulation delivery.

A lesion anatomy study indicated that task performance was related to lesion location rather than volume. In addition, variability in patients' response to treatment was observed and linked to lesion profile, revealing that damage to specific brain regions caused lack of tDCS response.

Finally, a concurrent tDCS-fMRI study was conducted to examine brain network response to tDCS. Brain stimulation did not affect local connectivity, but rather influenced functional connectivity within large-scale networks in the contralateral hemisphere. This finding emerged across groups using different analysis approaches, confirming its robustness.

This systematic behavioural and imaging investigation supports a role of tDCS to improve non-lateralised deficits of neglect, which could be harnessed in future clinical trials. Furthermore, it sheds light on network response to precise cortical targeting, revealing its widespread effect.

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List of abbreviations

ADHD	attention deficit hyperactivity disorder
ANOVA	analysis of variance
ADL	activities of daily living
B0	main static magnetic field
B1	magnetic field produced by the radio frequency coil
BET	brain extraction tool
BIT	behavioural inattention test
BOLD	blood-oxygen-level-dependant
CDT	clock drawing test
CIF	clinical imaging facility
CT	computer tomography
d'	d prime, or target sensitivity
DAN	dorsal attention network
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
DTI	diffusion tensor imaging
DWI	diffusion-weighted MR imaging
EPI	echo-planar imaging
FA	flip angle
FD	framewise displacement
FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FoV	field of view
FSL	FMRIB software library
GRAPPA	generalised auto-calibrating partial parallel acquisition
GUI	graphical user interface
HASU	hyper acute stroke unit
H+	hydrogen
HC	healthy controls
ICA	independent component analysis

LC	locus coeruleus
LECN	left executive control network
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MCA	middle cerebral artery
MELODIC	multivariate exploratory linear decomposition into independent components
MNI	Montreal neurological institute
MRI	magnetic resonance imaging
NA	noradrenaline
NHS	National health service
PACS	picture archiving and communication system
RF	radiofrequency
ROI	region of interest
RECN	right executive control network
R-fMRI	resting-state functional magnetic resonance imaging
RSN	resting-state network
RT	reaction time
SLF	superior longitudinal fasciculus
SN	salience network
TBI	traumatic brain injury
tDCS	transcranial direct current stimulation
TE	echo time
TMS	transcranial magnetic stimulation
TPJ	temporoparietal junction
TR	repetition time
VAN	ventral attention network
VLSM	voxel-lesion symptom mapping
VSN	visuo-spatial network
WM	working memory

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Chapter I:
General Introduction

1 The study of attention

We constantly take information in from the environment. By deploying attention, we can usually do so without being overwhelmed by the amount of sensory data available to us at any time. At the broadest level, attention allows us to take notice of a particular facet of the environment, prioritise sensory input that matters whilst ignoring other information, as well as sustain our responses. In their seminal review of the neural bases of attention, Posner and Petersen delineated an influential framework with three basic subsystems that perform different but interrelated functions: detecting a signal for conscious (we would now say voluntary) processing, orienting to sensory events and maintaining a vigilant or alert state (Posner & Petersen, 1990).

The study of attention from the perspective of cognitive neuroscience is characterised by an effort to parallel descriptions of processes with descriptions at the anatomical level. Notably, already in Posner & Petersen's early account, attentional functions were considered to be carried out by discrete *networks* of anatomical areas that interact with domain-specific systems, rather than being a property of a single centre or a function of the brain as a whole (also see Mesulam, 1981; Petersen & Posner, 2012). An empirical investigation of the attention system has strongly benefitted from the study of patients, especially individuals with focal brain lesions affecting different components of these networks.

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1.1 Attention deficits following right-hemispheric stroke

In 2017, the estimated incidence of stroke was almost 13 million worldwide - a new stroke every 2.5 seconds (Global Health Data Exchange). As more people are surviving a stroke than ever before (Feigin et al., 2014), stroke has become a leading cause of disability (Adamson, Beswick, & Ebrahim, 2004).

Among the most common sequelae of focal brain injury are attentional deficits. The syndrome of (spatial) neglect, as inattention is usually termed in behavioural neurology (Heilman, Valenstein, & Watson, 2000; Husain & Rorden, 2003; Mesulam, 2000), affects 23% of all stroke survivors (Appelros, Karlsson, Seiger, & Nydevik, 2002; Pedersen, Jorgensen, Nakayama, Raaschou, & Olsen, 1997). Sufferers from both left- and right-hemispheric stroke are equally likely to show signs of neglect in the acute post-stroke stages, but quite rapidly this pattern becomes unbalanced, with right-hemispheric stroke patients forming the overwhelming majority of individuals affected in more chronic stages (Stone, Patel, & Greenwood, 1993; Stone, Patel, Greenwood, & Halligan, 1992; Stone et al., 1991). Neglect has consistently been documented to be more frequent (Bowen, McKenna, & Tallis, 1999) and severe (Denes, Semenza, Stoppa, & Lis, 1982; Ogden, 1985) following right- as compared to left-brain damage, with its occurrence ranging from 10% to 80% of right-hemispheric stroke (Vallar & Bolognini, 2014). The main reasons for such variability are the tests performed to make a diagnosis, and at what time point since the stroke the assessment is being carried out (for exhaustive accounts, see Bowen et al., 1999; Buxbaum et al., 2004; Pedersen et al., 1997).

The high prevalence of attentional deficits in this population is particularly important considering that a recent systematic review focusing on recovery from stroke found that visuospatial and executive functioning were the only predictors of clinical outcome 6-12 months post-stroke (Mole & Demeyere, 2018).

1.2 Clinical presentation of neglect

Neglect is an acquired neuropsychological syndrome commonly reported following right-hemispheric stroke (Parton, Malhotra, & Husain, 2004). Recently, the adjective 'multicomponent' has been introduced to capture the well-described heterogeneity of the condition: patients with neglect clinically present with an array of attentional deficits, which are frequently found together but that can nevertheless occur in isolation (Husain & Rorden, 2003). For instance, patients may show spatially lateralised deficits, characterised by a disparity in performance in the left vs. right hand side of the space, together with non-lateralised deficits such as a diminished ability to maintain attentional focus over time, irrespectively of position in space.

1.2.1 Lateralised deficits

The most studied and well-described sign of the neglect syndrome is the spatial bias, characterised by a reduced ability to attend to stimuli depending on their location in space. The syndrome indeed takes its name from the clinical observation that patients tend to neglect (i.e., fail to attend to) the contralesional side of space. Whilst on acute wards, a large proportion of patients with right-brain damage show some degree of this pathological behaviour (Stone et al., 1991). They often present with a marked ipsilesional deviation of their head and eyes, and appear to ignore events occurring in the contralesional space (Fruhmann Berger, Pross, Ilg, & Karnath, 2006).

On formal assessment (see Chapter II, Section 2.2.2), when asked by clinicians to find targets among distractors in a visual array, patients typically start looking for targets from the right hand side of the page and struggle to find their way to the left, omitting numerous targets located on their left, despite being given unlimited time.

1.2.2 Non-lateralised deficits

More recent investigations have discovered that, following right-hemispheric damage, patients also show deficits that are not spatially lateralised and therefore

affect both sides of the space (Corbetta & Shulman, 2011; Husain & Rorden, 2003; Manly, 2002; Robertson, 1993; van Kessel, van Nes, Brouwer, Geurts, & Fasotti, 2010). Patients have disproportionate problems with a number of non-spatial attentional measures; difficulties are typically demonstrated by comparing performance for stimuli located centrally or in the ipsilesional visual field to that of a control group (Manly, 2002). It could be argued that non-spatially lateralised deficits have received substantially less attention in the literature than spatial attention deficits, in spite of the fact that non-lateralised deficits seem to better predict the chronicity and functional disability of neglect (Van Vleet & DeGutis, 2013a).

Examples of well documented non-lateralised components of neglect are selective attention deficits (i.e., an extended 'attentional blink', Husain, Shapiro, Martin, & Kennard, 1997), spatial working memory deficits (Malhotra et al., 2005; Malhotra, Mannan, Driver, & Husain, 2004; Striemer, Ferber, & Danckert, 2013), non-lateralised planning deficits in drawing production (Smith et al., 2007) and, crucially to the purposes of this thesis, vigilant attention deficits (Husain & Rorden, 2003). The latter, a loss of non-spatially lateralised general ability to monitor the environment over time, will be discussed in more details in Section 2.

1.2.3 Interaction between spatial and vigilant attention

As described above, neglect is a neuropsychological syndrome of pathological (spatial and non-spatial) attention, with lateralised and non-lateralised attentional deficits. A number of researchers have proposed the intriguing notion that spatial and non-spatial components of the syndrome may not only co-occur, but also interact (Corbetta & Shulman, 2011; Husain & Rorden, 2003; Robertson, 1993; Robertson, Manly, Beschin, et al., 1997).

Evidence in support of an interaction between the two has accumulated in the literature concerning healthy individuals. In the normal brain, arousal levels have been found to shape behavioural performance across space. For instance, the

advantage in reaction times (RT) typically registered for stimuli flashed to the left visual field, can be abolished by a warning cue that boosts arousal levels (Whitehead, 1991). Correspondingly, the slight leftward bias showed by adults on bisection tasks, known as pseudoneglect (Nicholls, Bradshaw, & Mattingley, 1999), can be reduced or even reversed in conditions of low arousal, such as in sleep deprivation (Manly, Dobler, Dodds, & George, 2005), or following time-on-task manipulation (>1 hour of testing) (Benwell, Harvey, Gardner, & Thut, 2013). Interestingly, a population typically affected by diminished vigilant attention such as older adults (see Section 2.3), also seems to show an attenuated or even reversed pseudoneglect (Benwell, Thut, Grant, & Harvey, 2014).

These findings suggest that a diminution in alertness may be sufficient to produce a rightward shift in visual attention in the healthy brain, opening up the possibility that a similar process may underlie persistent neglect following right-hemispheric stroke. Vigilant attention impairments also seem to be disproportionately more common following right- as compared to left-hemispheric stroke (see Section 1.3.2). The higher persistence of spatial bias following right as compared to left-brain-damage could be indeed explained by the presence of generalised vigilant attention deficits that may slow down and interfere with recovery (Husain & Rorden, 2003; Robertson, 2001). In this account, vigilant attention and other non-lateralised deficits interacting with the spatial bias would be responsible for the persistence of neglect (Husain & Rorden, 2003; Robertson, 2001); on the other hand, when the vigilance system is not damaged, as in left-hemispheric stroke, the spatial bias would recover swiftly. If this view was confirmed, vigilant attention deficits would assume a core role in the pathogenesis of neglect.

In stroke, there is some preliminary evidence that spatial and non-spatial attentional impairments may be correlated. An association between vigilant attention deficits and performance on standard spatial attention tasks was first described in a group of 17 moderate-severe chronic patients (Hjaltason, Tegner, Tham, Levander, & Ericson, 1996). When Robertson and colleagues tested a larger group of patients (n=44), they found that performance on an auditory vigilance

task could predict performance on a range of conventional measures of the spatial bias (Robertson, Manly, Beschin, et al., 1997). Using a visual vigilance task, Malhotra and colleagues found that vigilant attention was particularly impaired in a subset of right-hemispheric stroke patients with spatial attention difficulties, as compared to individuals who did not manifest a spatial bias (Malhotra, Coulthard, & Husain, 2009).

A causal link between lateralised and non-lateralised attention impairments can stem from two research streams that demonstrate that it is possible to bidirectionally modulate the spatial bias by leveraging arousal and general attention levels. For example, the administration of a sedative drug resulted in the immediate re-emergence of spatial neglect symptoms in recovered patients (Lazar et al., 2002). Furthermore, dual tasks, which significantly tax attention and working memory, have been shown to induce a rightward shift in spatial attention in healthy individuals (Peers, Cusack, & Duncan, 2006) and patients (Bonato, 2012; Bonato, Priftis, Marenzi, Umilta, & Zorzi, 2010; Ricci et al., 2016; Robertson & Frasca, 1992).

Conversely, and critical to the current thesis, the non-lateralised component has also been experimentally modulated to reduce the spatial bias. For example, increased alertness induced by time pressure during task performance improved ability to detect leftward targets on a cancellation task (George, Mercer, Walker, & Manly, 2008). In addition, an increase in either phasic moment-to moment attention (induced by a warning sound presented before each trial) (Robertson, Mattingley, Rorden, & Driver, 1998) or tonic sustained attention (Robertson, Tegner, Tham, Lo, & Nimmo-Smith, 1995) temporarily helped patients overcome the spatial bias. Recent interventional work found that motivational stimulation obtained by reward manipulation also improved the spatial bias (Malhotra, Soto, Li, & Russell, 2013; Olgiati, Russell, Soto, & Malhotra, 2016) - an effect that may be potentially mediated by changes in arousal levels (Olgiati et al., 2016). Finally, recent studies suggested that the lateralised attention deficits exhibited by patients with neglect can be ameliorated by noradrenergic medications that boost the ability to sustain attention (Dalmaijer et al., 2018).

1.3 Neural basis of neglect

Neglect is most common following a stroke in the right middle cerebral artery (MCA) territory, which often results in extensive cortical and subcortical brain damage – the individual distribution of damage is likely to account for the well-documented clinical heterogeneity (Stone, Halligan, Marshall, & Greenwood, 1998).

1.3.1 Neural correlates of lateralised deficits

Classic anatomo-clinical correlation studies attempted to identify a critical region responsible for the most apparent problem of neglect, the spatial bias. A large number of areas within the right hemisphere emerged, amongst which the most commonly reported are the inferior parietal lobule (Mort et al., 2003; Vallar & Perani, 1987), the superior temporal gyrus (Karnath, Ferber, & Himmelbach, 2001) and the inferior frontal gyrus (Husain & Kennard, 1996). Other identified regions include the temporo-parietal junction, the subcortical nuclei (thalamus and basal ganglia), the anterior insula and the middle frontal gyrus (Karnath, Himmelbach, & Rorden, 2002; Vallar & Perani, 1987). As a disparate number of regions within the entire right hemisphere came up from these studies, it became clear that it would not be possible to draw a direct inference from a focal cerebral damage to the syndrome of neglect. Efforts to link specific neglect behaviours or subtypes (e.g., egocentric vs allocentric neglect, depending on the frame of reference; personal vs. extra-personal neglect, depending on the affected sector of space) with defined lesions followed (Bisiach, Perani, Vallar, & Berti, 1986; Halligan & Marshall, 1991; Marsh & Hillis, 2008; Verdon, Schwartz, Lovblad, Hauert, & Vuilleumier, 2009).

In parallel, other studies have explored a possible role for white matter fibers connecting frontal, parietal and temporal cortices in the pathogenesis of neglect (Bartolomeo, Thiebaut de Schotten, & Doricchi, 2007; De Schotten et al., 2011; de Schotten et al., 2005; Doricchi, de Schotten, Tomaiuolo, & Bartolomeo, 2008; Doricchi & Tomaiuolo, 2003; Mort et al., 2003; Verdon et al., 2009). Large clinical studies have confirmed that stroke topography is predominantly subcortical

(Corbetta et al., 2015; Kang, Chalela, Ezzeddine, & Warach, 2003; Wessels et al., 2006), with white matter damage typically involving a region adjacent to the lateral ventricle, where arcuate and the superior longitudinal fasciculus (SLF) run parallel in anterior-posterior direction (Bartolomeo et al., 2007; Doricchi & Tomaiuolo, 2003).

Recently, the neural underpinnings of neglect have been further reconsidered as stroke started to be conceptualised as a disorder of brain networks with broader localisation (see review by Corbetta & Shulman, 2011). In this account, symptoms of neglect arise as a result of a dysfunction in the interplay between brain regions that normally show tightly coupled activity. In healthy individuals, two large-scale frontoparietal networks responsible for attention have been identified, namely the dorsal attention network and the ventral attention network (Corbetta & Shulman, 2002). An important feature of these networks is that while the dorsal attention network is bilateral, its ventral counterpart is typically right-lateralised and its location coincides with the areas predominantly damaged in patients with neglect (e.g., the supramarginal gyrus and the superior temporal gyrus are most commonly involved). According to Corbetta and Shulman, damage to these right ventral regions would be directly responsible for non-spatial deficits, including vigilant attention deficits (Corbetta & Shulman, 2011). The spatial bias observed in neglect, on the other hand, would reflect a dysfunction of the dorsal network, and may occur despite the dorsal network being anatomically intact; instead, its dysfunction would be the consequence of a disrupted interaction between the lesioned ventral network and the dorsal network.
A network approach to stroke seems particularly valuable as it encourages a move towards a more phenotypical approach to the understanding of post-stroke changes in behaviour (Corbetta et al., 2015).

1.3.2 Neural correlates of vigilant attention deficits

Converging evidence has accumulated on the centrality of rightly lateralised fronto-parietal areas in maintaining a vigilant state (Posner & Petersen, 1990).

Lesion studies showed that, although RT in response to a stimulus are slower after virtually any brain lesion, right parietal lesions produce the greater slowing (Benton, 1986; De Renzi & Faglioni, 1965; Howes & Boller, 1975). Patients with right parietal damage showed decreased galvanic skin response to electrical stimulation, when compared to healthy controls and left-brain damaged patients (Heilman, Schwartz, & Watson, 1978; Valenstein & Heilman, 1984). Similarly, heart rate deceleration, normally expected in anticipation of a response, was not observed when patients with right hemispheric damage performed an attentional task (Yokoyama, Jennings, Ackles, Hood, & Boller, 1987). These studies suggested that patients with right-hemispheric pathology may have altered autonomic responses to stimuli.

In addition, Rueckert and Grafman found that patients with right frontal lesions, as compared to patients with left frontal lesions and healthy controls, were slower, missed more targets, and also presented with a steeper decline in performance over a 10 minutes discrimination task (Rueckert & Grafman, 1996). Koski and Petrides studied a sample of patients who had undergone unilateral resection from the frontal or anterior temporal lobe for the relief of medically intractable seizures (Koski & Petrides, 2001). When tested on a 30-min location cueing paradigm, neurosurgical patients with excisions from either the left or right frontal cortex showed longer reaction times, relative to patients with excisions from the temporal lobe and normal control subjects. In addition, patients with right frontal lobe damage showed an additional deficit in the form of a stronger time-on-task decrement across blocks of trials than all other patient groups or healthy controls. More recently, a study by Malhotra and colleagues showed that damage to the right posterior parietal cortex (PPC) was associated with a decline over 8 minutes in a discrimination task that required maintaining attention to spatial locations (Malhotra et al., 2009).

A large body of imaging studies have also supported correlational links between right hemisphere activation and behavioural measures of vigilant attention. In a

Positron Emission Tomography (PET) study by Pardo and colleagues, the researchers discovered an increase in blood flow in the prefrontal and superior parietal cortex, primarily in the right hemisphere, when healthy adults performed a vigilance task (Pardo, Fox, & Raichle, 1991). FMRI studies in healthy individuals also showed that activation of frontal and parietal cortices, mainly in the right hemisphere, was associated with vigilant attention performance. For instance, in a concurrent EEG-fMRI study, Foucher and colleagues found that arousal measures, as indexed by EEG low-frequency power, were positively correlated with the fMRI signal of the right dorsolateral prefrontal and superior parietal cortices (Foucher, Otzenberger, & Gounot, 2004). A recent meta-analysis of 67 fMRI studies identified different clusters that consistently showed task-evoked regional activity across a core network subserving vigilant attention in humans (Langner & Eickhoff, 2013): these included the dorsomedial, mid- and ventrolateral prefrontal cortex, anterior insula, intraparietal sulcus and temporoparietal junction and subcortical structures such as thalamus and putamen. Activations in the right hemisphere recorded by these studies overlap particularly closely with the ventral attention network from the previously discussed model by Corbetta and Shulman (Corbetta & Shulman, 2002).

It has also been proposed that vigilance right-lateralisation may be a function of task characteristics. One study by Helton and colleagues used functional near infrared spectroscopy (fNIRS) to indirectly measure cerebral oxygenation levels while participants were performing two versions (high and low salience) of a 12-minute discrimination task (Helton et al., 2010). During the low salience version of the task, the right-hemisphere dominance observed with easy-to-discriminate targets disappeared, and a more symmetrical activity pattern was observed. Characteristics of tasks used to measure vigilance will be discussed in Section 2.2.

Finally, imaging studies examining brain connectivity at rest in healthy individuals also corroborated a key role of the right hemisphere in supporting vigilance (for a recent review, see Fortenbaugh, DeGutis, & Esterman, 2017). Using a 268-node whole-brain functional parcellation, Rosenberg and colleagues showed that it is

possible to predict overall performance on a vigilance task using functional connectivity patterns obtained both during task execution and at rest (Rosenberg et al., 2016). This suggested that the pattern of activations was, at least in part, the result of intrinsic and stable connections that also emerged during rest. By using the connection patterns derived from healthy adults, the authors were also able to predict ADHD severity scores in over 100 children using their scans. The prediction ability of these connectomes further validates the importance of functional connectivity in supporting sustained attention ability. Critically, a network characterization of vigilant attention in a clinical population with pathological vigilance secondary to stroke has never been explored. More research into the neuronal circuits mediating pathological vigilant performance is needed to determine the brain networks mediating vigilant attention. This would represent a crucial step into understanding the mechanisms underlying this cognitive function and would help develop theories of clinical deficits characterised by vigilant attention impairments.

In sum, vigilance as a construct relates to a number of task features, and as such may be difficult to localise precisely. Nonetheless, multiple features of vigilant attention have been linked to anatomical regions and networks, particularly a right lateralised fronto-parietal network. These findings have been drawn from patient groups and also from healthy participants taking part in functional imaging studies. Having described the importance of studying vigilant attention in the context of right-hemispheric stroke, the vigilant attention literature will now be reviewed in detail.

2 Vigilant attention

The capacity to track events as they unfold over time is not interminable: when concentrating on a task, we struggle and make more errors as time passes. Failures to monitor the environment may produce mildly negative consequences or even catastrophic effects, if such failures happen under some circumstances. For instance, concentration lapses have been associated with motor vehicle and

operator-related train accidents (Barkley, Murphy, & Kwasnik, 1996; Edkins & Pollock, 1997; McCarley et al., 2004). It is therefore not surprising that heavy technological investments have been made to assist with our (attentional) limitations: a driver drowsiness detector is now installed in some cars, alerting sounds have been incorporated to vital signs monitors in hospitals and radar surveillance drones can be employed by security in vicinity of many airports.

Cognitive psychologists use the term vigilance, or vigilant attention, to describe our ability to maintain attentional focus for a prolonged period of time (Davies & Parasuraman, 1982; Parasuraman, Warm, & See, 1998). Early descriptions of performance trends in industrial environments (e.g., Wyatt & Langdon, 1932) were followed by the first rudimentary laboratory experiments that aimed to reproduce features of watch-keeping duties to examine changes in performance (Lindsley & Anderson, 1944; Mackworth, 1948). These investigations arrived at a time (of war) when it was suspected that the efficiency of British soldiers in detecting critical signals kept deteriorating with time due to overlong spells. In Cambridge, Mackworth devised the Clock Test, whereby participants sat in a wooden cabin watching a screen with a hand (a black pointer) of a blank-faced circumference typically moving around in small steps (one movement per second), with rare unpredictable 'double jumps' that had to be detected (Mackworth, 1948). By employing this paradigm, Mackworth counted an increasing number of lapses over the course of a 2 hours watch, and observed improvements following some experimental manipulations, with the provision of feedback on performance and the administration of a small dose of amphetamine being successful in impeding the decline in performance and keeping performance at the initial high level of accuracy.

The overall ability to detect signals - the *vigilance level* - has been ever since investigated using different experimental paradigms in humans (see Section 2.2 below). Some of these also allowed for the measurement of the so-called *vigilance decrement* (Mackworth, 1948; Parasuraman, 1979). This is characterised by either

a decline in detections of critical stimuli over time or an increase in RT in response to targets, and is considered by some researchers the best way to capture the very nature of vigilant attention (Mackworth, 1964; Parasuraman et al., 1998; See, Howe, Warm, & Dember, 1995).

2.1 Terminology specification

Research in this field has been characterised by vaguely defined terminology. The terms arousal, alerting and sustained/vigilant attention have often been used interchangeably in many contexts, especially in clinical settings and when interpreting electrophysiological findings (Sarter, Givens, & Bruno, 2001). These entities are most certainly intertwined, but they are not entirely overlapping and it would be useful to have an operational definition of vigilant attention that is specific and dissociable from other concepts, which translates into what tasks represent good measures of vigilant attention (Sarter et al., 2001).

The necessary physiological basis of any vigilant behaviour requires an activated forebrain, which depends on the availability of the neurotransmitter noradrenaline. This is primarily synthesised in a midbrain structure known as the locus coeruleus (LC), a small collection of neurons located in the pons which has projections throughout the cortex. The technical use of the term *arousal* implies a brain state, a general neuronal excitability that varies within the sleep-wake cycle (Posner, Gazzaniga, & Blakemore, 1975). This state of general wakefulness represents the basis of any attention performance. Changes in arousal levels can modulate task performance for a short period of time by top-down (internal factors) or bottom-up (stimulus driven) processes. The first process, tonic alertness, is typically assessed by simple RT to stimuli without a warning signal. Phasic alertness, on the other hand, is measured with changes in RT in response to a warning cue that precedes the target (Sturm et al., 1999; Sturm & Willmes, 2001). In a neat set of experiments in monkeys, Marrocco and Davidson showed that when NA availability is inhibited pharmacologically by the administration of a sedative drug such as clonidine (i.e., a NA release inhibitor), the facilitating effect of

a warning signal on performance can be abolished (Marrocco & Davidson, 1998). The same manipulation in humans, the 'clonidine challenge', is also able to significantly increase the number of 'lapses of attention' (defined as the number of RT > 1500ms) in a simple-discrimination task (Smith & Nutt, 1996); interestingly, noise was able to reverse the effect by increasing NA release back again.
In the literature, the term *alerting* is typically used to capture the phenomenological aspects of arousal, i.e., the feeling of being alert, as well as the properties of emotional, stressful or novel stimuli such as the warning noises used in the experiments described above, which have been shown to interact with performance (Sarter et al., 2001).

The ability to sustain attention over time, which is of particular interest to cognitive psychologists and to this thesis, necessarily depends upon integrity of such physiological NA input in the sleep-awake axis. Noradrenergic activation alone, however, does not account fully for this cognitive process, and maintaining vigilant attention over time implies arousal plus some order of cortical, cognitive processing to sustain goal-oriented behaviour (Oken, Salinsky, & Elsas, 2006). For instance, the above-mentioned detrimental effect of clonidine on arousal was modulated by task familiarity (i.e., in the direction of a greater sedative effect in individuals familiar with the task), suggesting a role of task novelty in mediating the effect of the drug (Coull, Middleton, Robbins, & Sahakian, 1995). When referring to the process of maintaining an arousal state over time, in the case of a protracted presentation of rare stimuli, the term *vigilant attention* will be preferred. In the literature, the term *sustained* attention is also at times used in this context, but technically sustained attention refers to the ability to produce prolonged responses to *any* task, no matter the effort on the individual, whereas *vigilant* attention taps in to the processes that allows a prolonged response in situations that lack the arousing element of an external challenge (Robertson & O'Connell, 2010; Sturm & Willmes, 2001). Such a distinction therefore emphasizes the cognitive effort for the subject (the so-called cognitive load) and the task properties: vigilant attention can thus be regarded as the ability to self-sustain mindful, conscious processing of stimuli whose repetitive, non-alerting qualities,

would otherwise lead to habituation and distraction by other stimuli (Robertson, Manly, Beschin, et al., 1997).

Arousal and vigilance can be considered different but closely related constructs. Empirically, changes in arousal are typically inferred from moment-to-moment fluctuations in RT or physiological measures such as pupil diameter (Hopstaken, van der Linden, Bakker, & Kompier, 2015; Joshi, Li, Kalwani, & Gold, 2016; Murphy, Robertson, Balsters, & O'Connell R, 2011), skin conductivity (Blakeslee, 1979; Frith & Allen, 1983) and EEG markers (Berka et al., 2007; Martel, Dähne, & Blankertz, 2014), which appear to be sensitive to the activity of the cortico-thalamic networks underlying the sleep–wake dimension. On the other hand, an interpretation in terms of vigilant attention is necessarily based on behaviour (i.e., detection rates and false alarm rates). A few examples of experimental tasks used to track vigilant behaviour are discussed in the next section.

2.2 Measures of vigilant attention

Classic paradigms for measuring vigilant attention require participants to monitor for rarely occurring signals amidst unchallenging, monotonous events (Sturm & Willmes, 2001). Different tasks are typically employed by different laboratories to measure vigilance in healthy and clinical populations. At the broadest level, these attentional tasks can be divided into simple detection tasks, whereby targets are presented without competing distractors, and tasks that require participants to discriminate between targets and non-target stimuli. Whilst progress has been made in developing vigilant attention assessments for specific clinical populations, there is no single widely accepted clinical assessment that is sensitive to vigilance impairments across the diverse array of patient populations - this is an important goal for future research in this field (Fortenbaugh, DeGutis, et al., 2017).

A popular visual detection task that measures vigilant attention is the Sustained Attention to Response Task (SART) (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997), which has been used in healthy and brain-injured populations to

track vigilance and, more recently, to explore how this can be modulated by the application of brain stimulation (Brosnan et al., 2018). The SART, and the likewise popular Stop Signal Task (Logan, 1994; Logan & Cowan, 1984), present participants with a stream of visual stimuli (e.g., numbers), and require quick continuous responses to all stimuli but one. This results in a button press almost every ~1.5 seconds. This type of task has attracted some criticism for its strong component of motor response inhibition; in other words, it is not clear to what extent it taxes vigilant attention vs. the ability to suppress a prepotent response (Stevenson, Russell, & Helton, 2011). It is therefore debated whether the sustained attention deficits described in these studies are really the result of true lapses in sustained attention, or they rather reflect difficulties with a fast, coordinated motor response.

An even stronger motor component is involved in tasks that claim to assess vigilance by asking participants to produce a pace by finger tapping (for instance, see Petilli, Trisolini, & Daini, 2018). In the absence of distractors, participants listen to a set of tunes and are required to keep a rhythm. Although appealing in terms of experimental set-up and task duration, clear interpretation of findings may be problematic, as it can be difficult to disentangle cortical fatigue from a more physical muscular component.

In other paradigms, known as go no-go tasks, participants are required to monitor a stream of stimuli and respond only to an infrequent *go* target. Different studies examined the effect of manipulating task characteristics such as event rate and signal discriminability on the temporal dynamics of the vigilance decrement in healthy individuals (e.g., see Parasuraman, 1979). An example of an influential paradigm is the Continuous Performance Test (CPT), whereby letters are presented on the screen one at a time, at a fixed rate, and participants respond by pressing a lever whenever the letter X appears whilst inhibiting a response with any other letter (Beck, Bransome, Mirsky, Rosvold, & Sarason, 1956). Using this task, researchers were able to distinguish healthy adults and individuals with generalised brain damage with almost 90% accuracy. Rueckert and Grafman also

used the CPT to assess patients with focal frontal damage of different aetiologies (excluding stroke) (Rueckert & Grafman, 1996). By comparing performance between the first and the second half of the task, they were able to elicit a vigilance decrement, indexed by a slowing down in RT over time that was specific to right-brain damaged patients. Interestingly, such performance decrement over time disappeared when a distractor-free task version was used. Other studies in right-hemispheric stroke cohorts have also failed to elicit a vigilance decrement using simple detection tasks that do not require discriminating targets from non-targets (e.g., Exp 1 in Malhotra et al., 2009). Malhotra and colleagues for instance re-tested the same sample of right-hemispheric stroke patients using a visual detection task that required to discriminate targets from distractors, and also incorporated a (non-lateralised) spatial component, i.e. visual stimuli displayed vertically in the middle of a computer screen (Exp 2 in Malhotra et al., 2009). This relatively short task (around 8 minutes) elicited an overall vigilant attention deficit (i.e., lower accuracy) and a vigilant decrement (decline in accuracy over time) in right-hemispheric stroke patients with neglect, as compared to patients without neglect. It is possible that, to carry out this task, a tight interaction between frontal areas involved in vigilance and more posterior parietal regions containing space representations, typically dysfunctional following a stroke that causes neglect, was key (Corbetta & Shulman, 2011). This opens up the intriguing possibility that by targeting the crosstalk between these regions, it could be possible to alleviate patients' symptoms.

Finally, other tasks have been used to assess patients' vigilance in the auditory domain. In the elevator-counting subtask of the Test for Everyday Attention (TEA) battery (Robertson, Ward, Ridgeway, & Nimmo-Smith, 1996) for instance, patients are required to maintain a count of tones as if they were ascending on a lift. In the Lottery subtest, they need to detect a 'winning number' in a stream of auditory stimuli and retrieve the two letters presented before that number. Although these tasks have the merit of being more ecologically valid, they exert greater demands on the subject in terms of working memory, which can also be affected by stroke, and might be particularly difficult for older individuals with hearing problems.

In sum, different paradigms have been used to examine vigilance, with specific features of these cognitive tasks which have been shown to affect performance. Paradigms may use different levels of stimuli degradation, present stimuli at fixation or incorporate a spatial component with stimuli distributed across the screen or require response to any targets vs present distractors alongside targets. Typically, inter-stimulus interval is kept constant., e.g. on the order of 2 seconds, to allow time to process the stimulus and respond, avoiding muscular fatigue (Petton et al., 2019). Such tasks characteristics are reflected in the perception of a monotonous unchallenging task.

The paradigm used in the current study (described in detail in Chapter IV, Section 3.3.1) was selected because it had already demonstrated ability to detect a vigilance decrement in the relevant clinical population, i.e., right-hemispheric stroke patients with neglect (Malhotra et al., 2009). It involved presenting visual targets alongside distractors to different spatial locations on the screen, at a fixed rate. In the present investigation, the duration of the original paradigm was extended, so to increase chances to observe a potential tDCS-induced modulation of the vigilance decrement.

2.3 Vigilant attention across the life span

A decline in vigilance has been described in the ageing population (Fortenbaugh et al., 2015). Mani and colleagues found that, among 19-82 year old adults who completed a brief CPT, task accuracy decreased as participant age increased, as indexed by the increased number of false alarms (Mani, Bedwell, & Miller, 2005). A recent large-scale study of over 10,000 participants showed a similar pattern, with an even more precise modelling of age-related differences: performance, with regards to target sensitivity, peaked in the early 40s and declined thereafter; RT slowed down in an almost linear fashion from mid-teens to 60s (Fortenbaugh et al., 2015). Other studies using different paradigms have yielded more evidence in favour of age-related decline. McAvinue and colleagues found that the lifespan trajectory of sustained attention, as measured with the SART, was best

represented by a U-shaped curve, with poor performance in childhood and the teenage years, a plateau during young to middle adulthood, and deterioration in older adulthood (McAvinue et al., 2012).

Other studies however failed to replicate an age-related difference in vigilant attention (Berardi, 2001; Davies & Parasuraman, 1982; Rogers, 2000; Staub, Doignon-Camus, & Bonnefond, 2012). For instance, a large-scale study that used the classic Mackworth Clock Task, did not find any evidence of vigilant attention deficits in older adults (Giambra & Quilter, 1988). The majority of the evidence however points towards a vigilance decline in older populations, with inconsistent results attributable to the different features of the paradigms used. The potential presence of age-related differences demands special attention when comparing data from individuals across the life span and pathological groups.

2.4 Vigilant attention in clinical populations

Many clinical conditions feature deficits in vigilance. Impairments have been described in developmental disorders such as Attention Deficit Hyperactivity Disorder (ADHD) (Barkley, 1992; Bellgrove, Hawi, Kirley, Gill, & Robertson, 2005), tauopathies (O'Keeffe et al., 2007), psychiatric conditions such as depression and bipolar disorder (Marotta et al., 2015), individuals with early-life trauma (Fortenbaugh, Corbo, et al., 2017), traumatic brain lesions (Robertson, Manly, Andrade, et al., 1997) and sleep disorders such as sleep apnoea (Engleman, Martin, Douglas, & Deary, 1994; Verstraeten & Cluydts, 2004) and narcolepsy (Valley & Broughton, 1983).

Vigilant attention appears to be very sensitive to focal brain damage too, with right-hemispheric stroke patients often presenting as distracted and disengaged, with severe and long-lasting impaired ability to maintain concentration on many daily activities (Robertson, 2001). Relatives of right-hemispheric stroke patients often comment that their family member easily “drifts off” and appears not to be listening when they are talking to them (Robertson, 2001). An impairment of

vigilant attention can also manifest in the tendency to lose concentration whilst reading a book or following a TV program, or when engaging in other prolonged activities such as therapy sessions. This is particularly relevant considering that patients are offered care packages that normally involve 1-hour therapy sessions on neurorehabilitation wards. Their ability to concentrate and engage in such sessions is however not typically formally assessed. When this is tested experimentally, using one of the above-mentioned experimental paradigms, right-hemispheric stroke patients typically show signs of diminished physiological arousal and vigilant attention. Thus, it is potentially of great importance to assess and monitor vigilance attention, as vigilance impairments could be targeted specifically, enabling patients to fully benefit from rehabilitation.

2.5 Modulating vigilant attention

Neglect is associated with poor motor recovery, higher disability and generally with poor response to rehabilitation (Buxbaum et al., 2004; Cherney, Halper, Kwasnica, Harvey, & Zhang, 2001; Katz, Hartman-Maeir, Ring, & Soroker, 1999; Paolucci, Antonucci, Grasso, & Pizzamiglio, 2001). Compared to other patient groups with similar lesion volume, patients with neglect consistently score lower at both admission and discharge measures of functional ability and activities of daily living (Denes et al., 1982; Jehkonen et al., 2001; Kalra, Perez, Gupta, & Wittink, 1997). Hence, there is a pressing need to develop effective treatments for neglect. However, these patients are considerably resistant to many rehabilitation strategies when compared to other patients with acquired brain injury. Although the lateralised aspect of neglect recovers in the majority of patients, and appears to improve faster than other general attention deficits (Karnath, 1988), it represents the key target in the majority of treatment studies, following on from its striking manifestations and its defining role in diagnosis (for a recent review, see Azouvi, Jacquin-Courtois, & Luauté, 2017). Experimental evidence exists for different techniques, but there are no convincing randomised trials demonstrating clinically significant improvements as yet – a difficulty that is most likely attributable to patient heterogeneity.

There is now increasing interest in the development of treatments that tackle non-spatially lateralised mechanisms, such as vigilant attention deficits. These are common, persistent and associated with post-stroke disability, and as such represent a potential rehabilitation target that has been under investigated (Robertson, 2001; Striemer et al., 2013). This approach would potentially benefit most patients, as these deficits affect nearly all patients with persistent neglect (DeGutis & Van Vleet, 2010). Treating sustained attention deficits may also transfer to other domains: by boosting patients' capacity to engage and take information in from the environment, patients could for instance derive greater benefit from physical and cognitive rehabilitation (e.g., physiotherapy and occupational therapy, but also visual scanning and prism adaptation training for spatial bias).

Current treatments that proved able to improve non-lateralised attention deficits include pharmacological treatment targeting the NA system, which have shown promise at enhancing performance following encephalomyelitis (Singh-Curry, Malhotra, Farmer, & Husain, 2011) and stroke (Dalmaijer et al., 2018; Malhotra, Parton, Greenwood, & Husain, 2006). In a randomised, double-blind, placebo-controlled, crossover study, Dalmaijer and colleagues found that the administration of a single dose of 2mg of the noradrenergic alpha-2A agonist guanfacine to a sample of 13 right-hemispheric stroke patients, significantly improved the total number of targets found on a cancellation task, with no difference between targets found on the left and the right side of the array (Dalmaijer et al., 2018).

Behavioural pre-trial manipulations have also shown to influence vigilance levels. In neglect, proof of principle studies showed that the presentation of an alerting signal (Finke et al., 2012; Robertson et al., 1998) or a cue previously associated to a monetary reward (Esterman et al., 2016; Olgiati et al., 2016) were able to improve task performance in patients, most likely via a boost in arousal levels.

A few studies have examined the use of training techniques designed to improve vigilant attention. DeGutis and Van Vleet trained participants to monitor hundreds of visual scenes. They demonstrated significant improvements on measures of visual search, midpoint estimation and working memory (WM) in a sample of 12 healthy adults, as compared to a control group (DeGutis & Van Vleet, 2010). A similar training paradigm was then adapted for use in a sample of eight moderate to severe patients with neglect, who were asked to track the environment to discriminate auditory cues. After completing nine training days (three rounds of 12 minutes per day), patients showed significant improvement in several, untrained measures of lateralised and non-lateralised attention, as compared to a control group (Van Vleet & DeGutis, 2013b).
Robertson and colleagues trialled a sustained attention training with 8 stroke patients, during which they aimed to improve endogenous control by providing them with a mechanism to self-boost alertness periodically (Robertson et al., 1995). It should be noted that this approach, however, might not be applicable to the most severe cases or in the more acute stages post-stroke because it requires some degree of awareness for the condition that is not always present.

One type of experimental manipulation that can be used to modulate behaviour involves the application of non-invasive brain stimulation. Interestingly, a few recent studies have indeed reported a modulation in attention following application of non-invasive brain stimulation in patients with neglect; these are discussed in the next section.

3 Transcranial Direct Current Stimulation

Transcranial Direct Current Stimulation (tDCS) is a non-invasive brain stimulation technique which allows the modulation of spontaneous brain network activity (Bikson et al., 2016). By promoting changes in cortical excitability, the application of a low-intensity (typically 0.5-2 mA) (Zaghi, Acar, Hultgren, Boggio, & Fregni, 2010) constant electrical current can drive brain activity and facilitate/interfere with a cognitive function (see review by Kolb, Teskey, & Gibb, 2010; see

comprehensive technical guide by Woods et al., 2016). Given its potential to affect plasticity (Kolb et al., 2010), tDCS may be a particularly useful tool to enhance cognition in the context of neurorehabilitation (Vallar & Bolognini, 2011). Certain characteristics, such as being non-invasive, relatively cheap and mostly well-tolerated, with transient and mild side effects, make tDCS particularly appealing for clinical research aiming to augment cognitive functions following a brain insult. With evidence in healthy individuals accumulating (Polania, Nitsche, & Ruff, 2018), the use of tDCS to improve post-stroke outcomes has become a popular area of exploration, particularly in the motor (Stagg & Johansen-Berg, 2013) and language domains (Fridriksson et al., 2018; Holland & Crinion, 2012).

Nonetheless, its use is controversial (e.g., see Filmer, Mattingley, & Dux, 2019; Horvath, Forte, & Carter, 2015; Opitz, Falchier, Linn, Milham, & Schroeder, 2017) and there are ongoing debates regarding its clinical effectiveness (Cappon, Jahanshahi, & Bisiacchi, 2016; Kekic, Boysen, Campbell, & Schmidt, 2016). Critiques of this field stem around different key points. A first concern is whether any amount of current at all effectively reaches the brain (Shen, 2018). Animal studies have however showed that current intensities typically used in humans can produce a measurable physiological change. My work was set up to apply 1mA transcranially, which has been shown to induce 0.4 V/m in previous works (Huang et al., 2017), inducing measurable physiological changes (e.g., see Kar, Duijnhouwer, & Krekelberg, 2017). This was confirmed by my computational modelling, presented in Chapter 3, Figures 3.2, 3.3. and 3.4.

A second common concern relates to the unknown mechanisms of action of tDCS. This field of research is however expanding, with animal and multimodal imaging studies looking at the immediate/concurrent effect of stimulation (Stagg, Antal, & Nitsche, 2018). More effort to increase our understanding of how tDCS modulates the cortex is welcome, and accordingly, in the present work I assessed the effects of a current intensity of 1mA on behaviour and on networks by using tDCS simultaneously with neuroimaging. Having said that, whilst a situation where

mechanisms of actions are unclear is not ideal, it does not invalidate the use of tDCS (Filmer et al., 2019).

Another point for concern is the reproducibility of the tDCS-effects (Horvath, Carter, & Forte, 2014). This has been linked to the large parameter space of tDCS, which may be difficult to capture in systematic reviews, and suboptimal study designs used in the early stages of the field (Filmer et al., 2019). A recommendation here, which was incorporated into the present work, was to carefully power a study for the specific research question and use robust crossover methodology. Also, I aimed to replicate the beneficial effect of prefrontal tDCS found in younger adults in a stroke population disproportionally affected by vigilance deficits, using a brain stimulation protocol and an experimental paradigm optimised for clinical application.

Finally, and linked to this point, there is a methodological concern around the large inter-individual variability of the effect, i.e., some people show a response and other people do not show changes. By learning about potential sources of variability (for instance, see Chapter III, Section 2.7), we would be able to increase our understanding of how tDCS influences mental activity. In this work, I have planned to link age and lesion anatomy with behavioural response to tDCS.

3.1 tDCS for lateralised deficits

Most studies have focused on tDCS-induced modulations of what is considered to be the core deficit of neglect, the lateralised attentional component that causes difficulties in orienting towards the contralesional hemispace (see Appendix I for a summary of available evidence on the use of tDCS for lateralised and non-lateralised attentional deficits of neglect). There is preliminary evidence that tDCS can improve the spatial bias when applied as a stand-alone technique and in synergistic application with other therapies (Olgiati & Malhotra, 2020; Zebhauser, Vernet, Unterburger, & Brem, 2019). However, to-date there is no high-quality evidence that tDCS is an efficacious therapy in neglect.

3.2 tDCS for non-lateralised attention

One further approach towards tDCS in patients with neglect is to target non-lateralised large-scale brain networks involved in the control of attention (Corbetta & Shulman, 2011). This could help to modulate the common and more persistent cognitive deficits associated with neglect, which represent a potential rehabilitation target that has been underinvestigated (Robertson, 2001; Striemer et al., 2013).
To date, there has not yet been any systematic evaluation of whether there is a therapeutic role for tDCS in the modulation of non-lateralised attentional deficits following stroke. A very few studies directly examined whether spatial working memory and vigilant attention deficits caused by a focal brain injury are affected by tDCS.

Available evidence supports a role of prefrontal stimulation in working memory augmentation. For instance, an improvement in measures of spatial working memory following the delivery of tDCS, with anodal-electrode over the right dorsolateral prefrontal cortex (DLPFC), has been described in healthy individuals (Ruf, Fallgatter, & Plewnia, 2017) and in people with schizophrenia (Schwippel et al., 2018). Furthermore, there is evidence that verbal working memory can be improved in patients with right hemisphere stroke by 30 minutes of tDCS application to the left DLPFC (Jo et al., 2009).

More recently, studies have suggested that vigilant attention can also be boosted by applying tDCS to the prefrontal cortices in healthy younger (Nelson, McKinley, Golob, Warm, & Parasuraman, 2014) and older individuals (Brosnan et al., 2018), as well as individuals who have suffered a traumatic brain injury (Kang, Kim, & Paik, 2012), opening up the possibility that this could also be implemented for stroke patients. Kang and co-workers reported a positive effect of tDCS on a go/no-go task following a 20 minutes tDCS application with anodal electrode over the left DLPFC in a sample of 10 stroke patients (Kang, Baek, Kim, & Paik, 2009). Interestingly, in this offline paradigm, active tDCS was found superior to sham in

boosting accuracy (but not RT) at 1-hour and 3-hour post-stimulation timepoints, with no detectable changes immediately after stimulation. However, this go/no-go task had minimal requirements in terms of vigilant attention (the task was very brief itself, comprising just 30 trials) and working memory (respond to number '1' but not number '2').

In another proof-of-principle study, Park and colleagues chose an unconventional tDCS set up, with two stimulators connected to two anodal electrodes positioned over scalp coordinates F3 and F4 and two cathodal electrodes placed on the nondominant left arm, one next to the other (Park, Koh, Choi, & Ko, 2013). The intended outcome of this stimulation montage was the simultaneous targeting of bilateral DLPFC. They applied 30 minutes of real/sham tDCS daily, for 5 days a week until discharge, during a computer-assisted rehabilitation program targeting attention and memory. In a between-subjects design that included 11 patients, they reported an improvement in performance post- vs pre-treatment on a CPT in the tDCS group only. The choice of a between-groups design, with sub-acute patients affected by either right- (n=7) or left-hemispheric (n=4) lesions, is critical: the two groups (real and sham tDCS) started from a significantly different auditory vigilant attention baseline, which may have influenced the result of the study.

Both these studies explored, for the first time, the potential of tDCS, both alone and in combination with cognitive rehabilitation, to influence general attention levels in patients with stroke. However, the inclusion/exclusion criteria were broad, and patients with lesions throughout the brain were recruited, even though attentional problems are typically persisting and disproportionately severe following right-hemispheric stroke (Bowen et al., 1999; Denes et al., 1982; Ogden, 1985). Both sets of investigators used tasks with non-lateralised stimuli presented in the middle of the screen but did not test for the presence and severity of lateralised attention deficits, which might have been useful in characterising the clinical population.

The present thesis represents an effort to develop this line of investigation and systematically explore whether there is a therapeutic role for tDCS in the modulation of non-lateralised attention deficits following stroke.

4 Experimental strategy

The overarching aim of the present investigation is to explore the potential of precise brain stimulation in augmenting vigilant attention post-stroke. The methodology used on stroke wards to screen patients for attentional deficits is reviewed in Chapter II; in addition, imaging methods and the complete lesion mapping procedure is detailed. In Chapter III, a novel brain stimulation procedure, designed for application alone and in combination with resting state connectivity, is presented. Different experimental studies will then be described.
First, I will explore whether targeted tDCS applied to the right prefrontal cortex can modulate vigilant attention in healthy adults across the life span (Chapter IV). Then, I will move on to examine the efficacy of tDCS to modulate vigilance in a stroke population affected by attentional deficits (Chapter V). In Chapter VI, I will explore the anatomical correlates of vigilant attention and tDCS responsiveness. Furthermore, a concurrent tDCS-fMRI study conducted in healthy adults and patients with neglect will be presented. This allowed for an investigation of network response to brain stimulation. Finally, Chapter VII provides a summary of my findings, and explores future directions that research in this field could take in the light of my original findings.

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Chapter II: General Methods.
Patients screening and brain imaging

1 Introduction

In this chapter, the methods used to identify and assess right-hemispheric stroke patients with neglect are described. In addition, methods used to obtain anatomical and functional brain images and create lesion maps for the purpose of this thesis are defined.

2 Acute cognitive screening

The overarching aim of this thesis is to explore the potential of a theoretically driven approach to alleviate vigilant attention deficits following right-hemispheric stroke. Although the ability to sustain attention may seem to be affected by lesions affecting numerous brain regions, right hemisphere patients with neglect are a population that has been demonstrated to be severely and disproportionally affected by vigilant attention deficits, following on from vigilance lateralisation to the right hemisphere of the brain (Chapter 1, Section 1.3.2). Therefore, the work in the current thesis focussed around this patient group. Having had neglect at stroke onset impact on the rehabilitation process, as we know that neglect is associated with poorer functional outcome post-stroke (Mole & Demeyere, 2018).

Neuropsychological screening of acute patients on stroke wards was performed to identify a group of potential research participants who were likely to exhibit specific features of the neglect syndrome in the chronic stage. Studies have showed that individuals with neglect present with an array of attentional difficulties that include both lateralised and non-lateralised deficits, with the latter typically persisting in chronic stages (see Chapter I, Section 1.2).

Given that neglect is such a heterogeneous condition, my screening strategy involved the use of a battery of tests to identify individuals with evidence of clear neglect acutely, as these patients were particularly likely to have persisting non-lateralised deficits (especially vigilant attention impairments) in chronic stages. Considering that there is no clear consensus on what tests should be included in an ideal battery to detect for the presence of neglect or to indicate its severity (Li & Malhotra, 2015), I selected a set of tests that allowed for the identification of neglect in the personal and extra personal space as well as representational neglect. In addition, patients were examined for the presence of primary sensory deficits and additional neglect-related phenomena such as extinction.
By using a comprehensive clinical battery to probe neglect, it was ensured that the majority of neglect patients could be identified.

The following categories of patients were excluded:

- Patients whose stroke was not confirmed radiologically;
- Patients with previous clinical strokes;
- Patients with a history of other neurological disorders, such as traumatic brain injuries (TBI) and neurodegenerative diseases. In cases where such a diagnosis had not been made, individuals were not recruited when there was a strong clinical suspicion of an underlying degenerative condition (e.g. patient not living independently, in a care home with full package of care prior to admission);
- Patients with medical comorbidities other than stroke, such as oncological diseases;
- Patients with a history of major psychiatric disorders, such as psychotic disorder and major depression;
- Patients whose limited English would have required the use of a translator to conduct the assessment and the research session, with the exception of Italian and Spanish speakers whom I was able to assess autonomously.

2.1 Recruitment process

After obtaining approval from the local ethics committee (i.e., the London Central Research Committee), I personally screened 69 patients who presented with signs and symptoms of acute right-hemispheric stroke. They were recruited from the hyper-acute stroke unit (HASU) on 9North and the stroke wards on 9West, 9South and the Neuro-Rehab Unit at Charing Cross Hospital (London), between September 2015 and July 2017. Potential cases of neglect were identified by monitoring hospital admissions on stroke wards and by attending weekly multidisciplinary team meetings. My sample of screened patients included individuals with acute and sub-acute stroke of both sexes (F= 31) and across the life span (mean age 64.69 years old, SD±15.31, age range 22-96 years old). I then trained and supervised another member of the research team, who continued to carry out a brief cognitive assessment on the wards when I was unable to - 14 out of 27 patients recruited to the studies described in this thesis came from this latter group.

2.2 Screening battery

Identified individuals were approached on the ward and potential participation in research was discussed. When verbal consent was obtained, a clinical assessment specifically developed for screening on stroke wards was carried out. This bedside clinical assessment required 40-50 minutes of time with each patient - when the visit had to be suspended (e.g., because the patient was medically unwell, busy with therapies, had imaging or other examinations scheduled), an effort was made to complete the screening over consecutive days, as appropriate. However, not all patients were able to complete all the tests in the battery due to their acute medical condition, inability to follow task instructions or fatigue, or because they were discharged before the battery could be completed. A minimum of two neuropsychological tests were performed by all patients screened.

2.2.1 Neurological examination

A brief neurological examination was carried out to assess for the presence of any contralesional motor, somatosensory and visual field deficits (Anderson et al., 2005; Bisiach, Cappa, & Vallar, 1983; Bisiach, Vallar, Perani, Papagno, & Berti, 1986; Haerer, 1992). The results of the neurological examination are summarised in Table 2.1.

As part of the upper limb **motor** examination, patients were asked to hold their arms out horizontally and palms up for thirty seconds, eyes closed. Any sign of asymmetric strength deficit (e.g., arm drift or pronation) was recorded, with a score of 3 indexing maximum deficit (range 0-3). One patient out of 47 did not show any sign of motor weakness, 22/47 presented with mild deficits, 3/47 with moderate and 21/47 with dense motor impairments.

Visual fields (upper and lower quadrants) were tested using finger confrontation. Patients were instructed to fixate on the nose of the researcher, who performed a conventional manual confrontation test by finger wiggling whilst keeping the hands at about 20 degrees to the left and to the right. Patients had to report for the presence and location (unilateral left or right or bilateral) of any perceived movement. 15 patients out of 37 were able to detect all 10 contralesional stimuli, whereas 6/37 showed mild deficits, 2/37 moderate deficits, and 14/37 severe visual field deficits compatible with the presence of hemianopia and/or severe neglect.

Somatosensory processing was assessed with manual stimulation via brief and light pressure stimuli delivered with the fingertip on the back of the patient's hand. Patients had to report for the presence and location (unilateral left or right or bilateral) of any perceived touch. 16 out of 44 patients did not miss any contralesional touch, 8/44 had minor deficits and 7/44 moderate deficits; somatosensory loss was found in 13/44 patients.

For both visual and somatosensory assessment, stimuli were administered in two consecutive series. The first one comprised 10 unilateral left and 10 unilateral right stimuli. Any omission was recorded. If at least one contralesional stimulus was correctly reported, a second series comprising 10 bilateral and 10 unilateral stimuli was performed. This procedure allowed for the detection of visual/tactile extinction, a difficulty to report a relatively contralesional target in presence of a competing stimulus. The clinical phenomenon of sensory extinction, indexed by a difference between unilateral left and bilateral stimuli >20% (Bisiach et al., 1983; Bisiach et al., 1986), typically presents alongside neglect but can nevertheless occur in isolation, sometimes persisting when other manifestations of spatial bias have recovered (Li & Malhotra, 2015). Visual and tactile extinction were found in approximately half of the patients tested.

NEUROLOGICAL EXAMINATION	**Patients with deficit/patients tested (% affected)**
Upper limb weakness	46/47 (98%, severe in 46% of them)
Visual field deficit	22/37 (59%, severe in 64% of them)
Visual extinction	15/28 (54%)
Somatosensory deficit	28/44 (64%, severe in 46% of them)
Tactile extinction	19/35 (54%)
Anosognosia for - Condition (stroke) - Motor deficit - SS deficit - Visual deficit	 2/46 (4%) 11/45 (24%) 14/43 (33%) 14/35 (40%)
Somatoparaphrenia	3/27 (11%)
Alien Hand Syndrome	3/38 (8%)

Table 2.1: Summary of results of neurological screening on stroke wards
Numerator indicates the number of patients with a lateralised deficit on each task; denominator equates to the number of patients who performed the task; percentage of patients affected in parenthesis.

As part of the neurological interview, patients were also asked whether they knew the reason why they were in hospital - this allowed the identification of individuals that were aware and able to tell they had a stroke (44 patients, although two of them knew they had a stroke but said they were not concerned about this and asked to go home), and patients who lacked awareness into the condition (2 patients). Patients were then asked if they had noticed any change since the stroke. At this point, any sign of unawareness specific for the motor, visual and somatosensory impairments (i.e., **anosognosia**) was recorded on a 0-3 scale, with 0= disorder spontaneously reported by the patient, 3= no acknowledgment of the deficit, not even after demonstration (Bisiach et al., 1986). The number of patients with anosognosia in this sample is reported in Table 2.1.

The presence of **somatoparaphrenia** was explored by asking patients questions about any experience of reduced ownership for their left hand since the stroke:

- With examiner pointing to the patient right hand, 'Whose hand is this?'
- With examiner pointing to the patient left hand, 'Whose hand is this?'
- With examiner pointing to their own left hand, 'Whose hand is this?'

The presence of **alien hand** phenomena was explored by asking about any involuntary movement of the left hand (if not plegic), and any feeling of reduced ownership (e.g., see Olgiati et al., 2017).

2.2.2 Neglect battery

Visuo-spatial attention can be assessed using different tests, which tap into related but different mechanisms (see Figure 2.1 for examples of tests performed on the ward by patients with neglect). Task performance for a given patient may vary, depending on what test is used to make a diagnosis of neglect and what indices are being used to define impairment (Azouvi et al., 2006).
To test for the presence of neglect in the peripersonal space, performance on three traditional paper-and-pencil tasks was examined: two cancellation tasks and a line bisection task, each presented on a landscape oriented A4 sheet of paper aligned

with the mid-sagittal plane and kept in position on a table or a overbed table (only for when the patient was bedbound) using adhesive tape. In addition, two drawing tests were used.

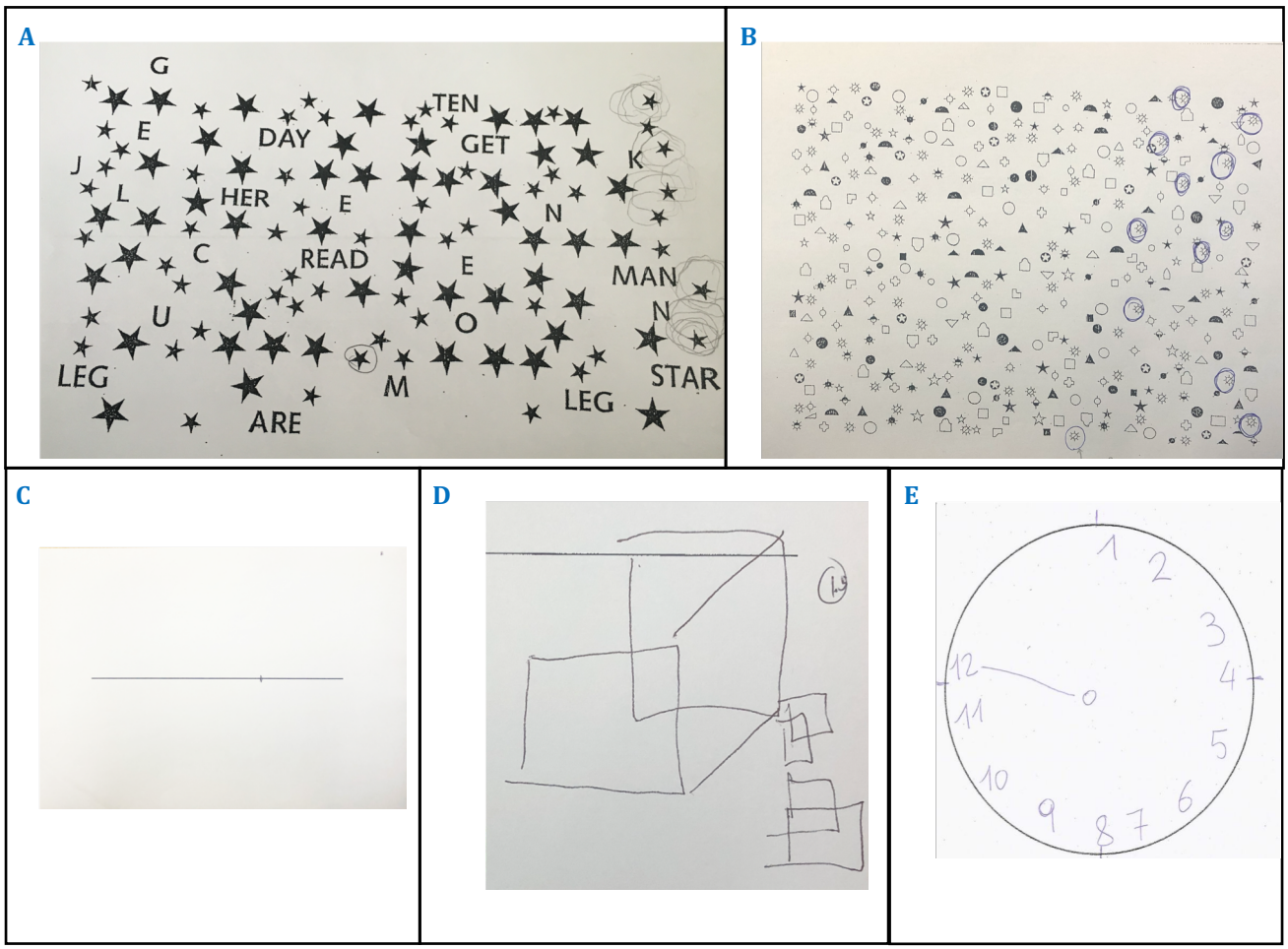


Figure 2.1: Examples of the lateralised bias in neglect, with asymmetries between left and right hemispace
Star cancellation test (A), Mesulam cancellation task (B), line bisection test (C), copy of a cube from the BIT (D) and clock drawing test (E) performed by acute and sub-acute neglect patients screened on stroke wards.

2.2.2.1 Cancellation tasks

Cancellation tasks typically requires patients to decide whether every stimulus on a page is a target or a non-target, and cross out only the former.

The **star cancellation test** requires marking with a circle all small stars distributed amid distractors (i.e., letters, words and big stars) across an A4 sheet, landscape oriented (Figure 2.1, A) (BIT battery by Wilson, Cockburn, & Halligan,

1987). When screening on the ward, one small star in the centre was used for demonstration, and patients were instructed to mark all remaining 55 targets and let the examiner know when they could not find any more. No time limit was imposed. Score: Number of targets correctly identified, 55=no deficit, 0=max deficit.

The **Mesulam cancellation task** requires identifying 60 symbols distributed across the space (30 on the left and 30 on the right) on a field of distractors (Figure 2.1, B) (Mesulam, 1985). Compared to the star cancellation task, it has a denser array with more targets and more distractors. Hence, this test is potentially more challenging for patients and may reveal a greater degree of impairment (Kaplan et al., 1991). A central symbol was marked by the examiner. Score: Number of targets correctly identified, 59=no deficit, 0=max deficit.

Individuals were identified as having a pathological spatial bias on cancellation tasks when they showed an asymmetric score between targets accurately detected on the right vs on the left; a difference ≥5% was considered an indicator for the presence of neglect.

2.2.2.2 Line bisection

The **line bisection test** requires patients to set the midpoint of 6 horizontal black lines (2mm thick) of different lengths (10cm, 15cm and 25cm) printed in the centre of an A4 sheet, landscape oriented, and presented in a random-fixed order (Figure 2.1, C) (Fortis et al., 2010). Score: The length of the left-hand side of each line (i.e., from the left end of the line to the landmark) was measured to the nearest millimetre. This measure was then converted into a standardised score using the following formula (Rode, Michel, Rossetti, Boisson, & Vallar, 2006):

$$\frac{(\Sigma\, measured\ left\ half\ -\ \Sigma\, objective\ half)}{\Sigma\, objective\ half} * 100$$

This transformation yields positive numbers for rightward displacements and negative numbers for leftwards displacements. Any positive score was considered an indicator of neglect - this corresponds to a value above 99% confidence interval [-2.3218, -0.0982] in an age-matched control group (n=65, mean=-1.21, SD=3.48) (Corbetta, 2008).

2.2.2.3 Drawing tests

A **drawing test** from the Behavioural Inattention Test (BIT) (Wilson et al., 1987) was included in the battery. An A4 sheet of paper was aligned, portrait oriented, with the mid-sagittal plane of the patient's trunk. On the paper, three simple black ink figures (a star, a cube and a daisy) are displayed on the left, and patients were asked to copy them in the space provided on the right (e.g., see Figure 2.1, D). Although drawing tests may display some remarkable manifestations of neglect, they are not very sensitive on their own (Azouvi et al., 2002) and are difficult to score in a graded manner (Parton, Malhotra, & Husain, 2004). Omissions or gross distortions on the contralesional side were considered indicative of neglect.

Patients' ability to draw from memory was assessed using the **Clock-Drawing Test** (CDT), which can elicit striking manifestations of representational, or imagined, neglect (Figure 2.1, E). In this task, patients were given a sheet of paper with a pre-drawn circle on it and were asked to draw the numbers as seen on a clock face. Performance was assessed by looking at the number of omissions in number sequencing and the symmetry of the numerals on the clock face. Abnormal drawings produced by patients can be characterised by visuospatial errors such as an abundance of empty space and misplacements on the left-hand side of the clock face. However, it should be noted that a substantial proportion of patients who manifest neglect on other tasks show no impairment when drawing clock faces. This heterogeneity in performance and the evidence that the impairment does not correlate with neglect severity as measured by other tasks, represent a challenge to the use of the CDT as a routine screening tool for neglect (Ishiai, Sugishita, Ichikawa, Gono, & Watabiki, 1993). Score: 1 point given for each number in the correct position, 0 given for each omission or translocation of an element from one

side to another; 12=no deficit, 0=max deficit (Mancini, Bricolo, Mattioli, & Vallar, 2011). Upon comparison with the mean score of 148 neurologically unimpaired (mean age=61.89, SD±11.95, range 40-89), which was 11.55 (SD±1.17), a score lower than 9 was considered suggestive of a pathological performance (Mancini et al., 2011).

2.2.2.4 Personal neglect

Personal neglect was assessed by asking patients to touch six contralesional body parts with their non-paretic ipsilesional hand; the accuracy of each attempt was graded (Bisiach et al., 1986). Score: 0-18 (18=unimpaired performance; 0=maximum deficit).

A summary of patient performance on neglect measures is shown in Table 2.2.

NEGLECT battery	**Patients with deficit/Patients tested (% affected)**
Star cancellation	28/46 (61%)
Mesulam cancellation	22/32 (69%)
Line bisection	38/44 (86%)
BIT shape copy	25/34 (70%)
Clock drawing task	12/34 (35%)
Personal neglect	12/43 (28%)

Table 2.2: Summary of neglect screening on stroke wards
Numerator indicates the number of patients with a lateralised deficit on each task, denominator equates to the number of patients who were able to perform the task, percentage of patients affected in parenthesis.

As discussed, there is no clear consensus on what represents conclusive evidence for the presence of neglect, and what tests should be used to track any residual deficits. For the purpose of recruiting individuals with neglect suitable for the

research presented in this thesis, I used an operational definition of neglect as follows: patients were defined as having neglect if they demonstrated evidence of a lateralised deficit on two or more tests, or on a single task in conjunction with a right-sided start on the cancellation tasks (Azouvi et al., 2002; Stone et al., 1991). Most of the patients (80%) screened by me at Charing Cross Hospital presented with clinical signs and symptoms of neglect. It should be noted that these numbers are not comparable with epidemiological studies: they do not come from a cohort of consecutive patients admitted to the hospital; instead they are derived from a programme specifically designed to identify this patient group for experimental testing.

In sum, patients were tested using standard measures of spatial bias in an acute/post-acute stage after stroke; they would then be re-tested in a chronic stage of their illness (Figure 2.2). The expectation was that spatial bias would have resolved or improved when tested chronically >90 days post-stroke, and vigilant attention deficits would be present at varying degree – these would be targeted by the tDCS. All patients recruited for the studies described in this thesis had: i) evidence of a first clinical stroke in the right hemisphere of the brain, of varying size; ii) evidence of an attentional impairment in the acute stage, of varying entity. Clinical characteristics and recovery rates for lateralised manifestations of neglect at time of participation are to be found in Chapter V, Section 3.1 'Participants'.

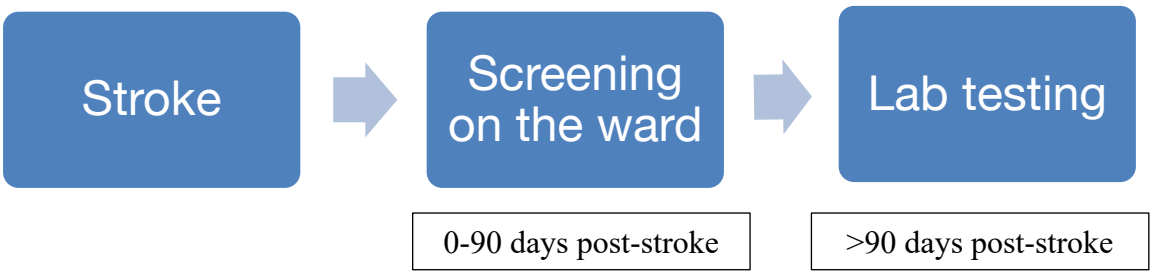


Figure 2.2: Timeline of neglect assessment
Screening on the ward aimed to identify individuals at risk of developing persisting vigilant attention difficulties. Re-testing in chronic stages was performed as a secondary outcome measure, to explore for any potential effect of offline tDCS on the lateralised component of the syndrome.

3 Brain imaging

As part of standard clinical care, patients would all have undergone a routine clinical computerised tomography (CT) and often a magnetic resonance imaging (MRI) brain scan during their admission at Charing Cross Hospital, with the purpose of confirming a diagnosis of stroke. In addition to this, patients who took part in the imaging study presented in chapter VI underwent structural and functional MRI at the Clinical Imaging Facility, Hammersmith Hospital, at the time of participation. This provided structural anatomical imaging in greater detail than clinical acquisition protocols.

The basic properties of the MRI signal, and the procedure used to acquire MR images in healthy and stroke populations as part of the study described in Chapter VI, are described in the following sections.

3.1 MRI signal basics

The MRI signal comes from hydrogen atoms, commonly found in molecules of water in the human body, interacting with externally applied magnetic fields. Hydrogen ions (H+) have a nuclear spin and an associated magnetic field that will tend to preferentially align parallel and anti-parallel to the strong stable magnetic field of the MRI scanner (B_0). When a second oscillating magnetic field (B_1) is applied, a phenomenon called resonance takes place: the energy imparted by a radiofrequency (RF) pulse perturbs the H+ and makes them move through 90 degrees relative to B_0, called transversal plane (excitation); when the RF pulse is switched off, the H+ ions go back to the longitudinal plane returning part of the energy as radio signals (relaxation). This event can be recorded using an antenna, or coil, placed around the head.

One of the main advantages of MRI compared to other imaging modalities is its excellent soft tissue discrimination (Westbrook & Talbot, 2018). The tissue containing H+ determines the rates of relaxation, which underlie the different signal intensities seen on the MR image. Specific sequences are optimised to detect

optimal contrasts between different tissues, which helps defining anatomy and detecting abnormalities in the brain. An MR image has contrast if there are areas of high signal (hyperintensity – white in the image) and areas of low signal (hypointensity – black in the image); some areas have an intermediate signal (shades of grey in-between white and black) (Westbrook & Talbot, 2018). T1 contrast is an image where fat is hyperintense and water is relatively hypointense because TR (Repetition Time, or time between successive excitation pulses applied to the same slice) and TE (Time to Echo, or time between the excitation pulse and the signal read-out at the receiver coil) are short enough to not allow full realignment to the scanner primary magnetic field. T2 contrast, on the other hand, is an image where fat is hypointense and water is relatively hyperintense because TR/TE are long enough to allow full dephasing.

Neuroimaging techniques can be used not only to describe anatomy, but also to characterize brain tissue function indirectly. In functional MRI (fMRI) studies for instance, changes in the blood oxygenation level dependent (BOLD) signal are measured and used as a proxy of brain metabolism. FMRI exploits the magnetic properties of blood. Haemoglobin is a molecule that contains iron and transports oxygen (which binds directly to iron) in the vascular system. When oxygen is bound (oxyhaemoglobin), the magnetic properties of iron are largely suppressed. When oxygen is not bound (deoxyhaemoglobin), the molecule becomes paramagnetic, which creates an inhomogeneous magnetic field in its vicinity (i.e., less MRI signal). At rest, venous blood contains an almost equal mix of oxyhaemoglobin and deoxyhaemoglobin. During exercise, metabolism is increased and more oxygen is needed. The cerebral vascular system increases blood flood to the activated area causing a drop in deoxyhaemoglobin, which result in an increase in signal intensity. BOLD images were acquired using echoplanar imaging (EPI) T2*-weighted sequences, which demonstrate high sensitivity to inherently inhomogeneous tissues. Functional MRI can be performed whilst participants are engaged in a cognitive task (task fMRI) or whilst participants are at rest (resting state fMRI), as in the work presented in this thesis and discussed in more details in Chapters III and VI.

Finally, Diffusion Tensor Imaging (DTI) is a sequence used to describe the movement of molecules of water due to random thermal motion. In some pathologies such as stroke, diffusion is restricted and the average diffusion in the tissue is reduced. Images can be acquired combining EPI with two large gradient pulses applied after excitation, designed to cancel each other out if spins do not move, whilst moving spins experience phase shift (Westbrook & Talbot, 2018). Diffusion sensitivity is controlled by the parameter 'b' which determines the diffusion attenuation by modifying the duration and amplitude of the diffusion gradient.

A detailed descriptions of the physics and basis of image reconstruction and manipulation techniques underpinning these methods is outside the scope of this thesis and can be found elsewhere (Attenberger, 2017; Kak & Slaney, 2001; Rosenfeld & Kak, 1982; Westbrook & Talbot, 2018).

3.2 MRI environment safety and tolerability

As part of the imaging study described in Chapter VI, an MRI exam (which included acquisition of T1, T2, fMRI and DTI) was conducted in healthy and clinical populations. On the day of testing, participants were asked about potential contraindications to scanning, as per the safety checklist provided by the Clinical Imaging Facility (CIF) (Appendix II). For patients with limited mobility, an MRI-compatible wheelchair was used to safely enter the room and reach the flat bed – assistance of two was then provided to transfer from the chair to the MRI table. Continuous monitoring during each MRI exam was performed visually via a shielded window and an MRI-compatible camera positioned in the room, and verbally via interphone that allowed two-way communication between participant and staff at the imaging console (Figure 2.3).

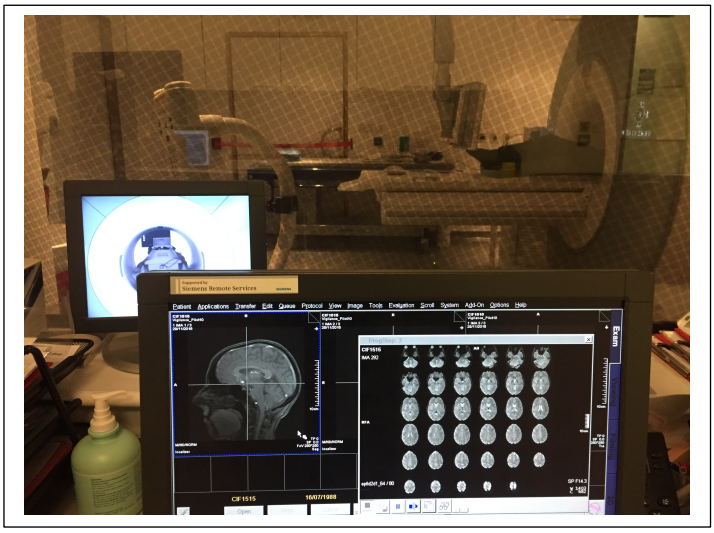


Figure 2.3: View from the imaging console at the Clinical Imaging Facility, Hammersmith Hospital

Despite efforts made to ensure that scanning (~1h protocol) was tolerated as well as possible (e.g., earplugs, extra-foams to improve comfort, frequent breaks etc.), two patients did not tolerate the noise of the T2 sequence (which was therefore not acquired) and three patients experienced difficulties towards the end of the protocol, such that they were unable to undergo the DTI run. Also, another patient preferred to split the protocol into two separate sessions.

3.3 Positioning and equipment set-up

Participants were placed on the nonferromagnetic table in the supine position, head first. Padding around the head was used to increase comfort and reduce head movement, improving data quality. This preparation was carried out by carefully checking that the electrodes positioned on the head and the MRI-compatible wires used to deliver brain stimulation intrascanner were kept in position. Every effort was made to keep participants' heads in the centre of the bore, before the receiver imaging coil was placed around the head. A call-buzzer was provided to hold (with the right non-affected hand in patients), and participants were instructed that a squeeze would start the alarm allowing the radiographer to stop the scanner. The couch was then raised and driven horizontally into the magnet bore. This movement was controlled from a panel situated on the front cone of the scanner

cover, which also allowed checking whether the region to be scanned was centred to a crosshair marker formed by lasers.

3.4 Imaging protocol

Scanning was performed at the Clinical Imaging Facility, Hammersmith Hospital, on a Magnetom Verio 3T MRI scanner from Siemens Healthcare (Erlangen, Germany) using a 32-channel head coil, which allowed full brain coverage. A mirror attached to the head coil and shifted on the mirror carrier just above the eyes allowed participants to see out easily, as soothing videos were played during anatomical runs on a monitor located inside the MRI room.
My protocol's timeline for sequence acquisition is displayed in Figure 2.4.

time →

localiser	fMRI + brain stimulation	T1	T2	fMRI + brain stimulation	DTI
00:13	10:06	5:03	5:34	10:06	11:15

Figure 2.4: MRI acquisition timeline (top row) and acquisition duration in minutes (bottom row)

3.5 Acquisition parameters

The scanner was operated in tandem with one of three expert MRI radiographers of the CIF who were familiar with the research protocol and the brain stimulation-fMRI set-up. In one imaging session, the following sequences were acquired:

3.5.1 Localiser

A set of 3-plane (axial, coronal and sagittal) low-resolution, large field-of-view localiser scan was obtained for plotting and prescribing the subsequent scans.

3.5.2 T1 MRI parameters

High-resolution T1-weighted structural images were acquired using a rapid gradient-echo (MP RAGE) sampling and used to aid registration of functional images and map lesions in patients.

The parameters used were: 1 mm^3 thick isotropic voxel, interleaved order of acquisition, sagittal orientation, repetition time (TR) 2300ms, echo time (TE) 2.98ms, inversion time 900ms, flip angle (FA) 9°, field of view 256×240mm, 160 slices, GRAPPA acceleration factor = 2 volumes, phase encoding direction A>P.
After acquisition, reconstructions in axial (co-planar with AC-PC) and coronal plain were performed, with image thickness 1mm, distance between images 3mm.

3.5.3 T2 MRI parameters

T2-weighted structural images were acquired using a 3D fast spin echo T2, to be used complementary to T1 to improve lesion localisation in patients.
The parameters used were: 0.9 mm^3 thick isotropic voxel, sequential order of acquisition, sagittal orientation, TR 3800ms, TE 494ms, FA variable, field of view 220x220mm, 176 slices, GRAPPA acceleration factor = 2 volumes, phase encoding direction A>P.
After acquisition, reconstructions in axial (co-planar with AC-PC) and coronal plain were performed, with image thickness 1mm, distance between images 3mm.

3.5.4 Functional MRI parameters

Functional MRI images were acquired in two runs (~10 minutes in-between runs) using a T2*-weighted gradient-echo, echoplanar imaging (EPI) sequence.
The following parameters were used: 3mm^3 isotropic voxel, distance factor 0%, interleaved order of acquisition, axial orientation, TR 2000ms, TE 30ms, FA 80°, field of view 192x192mm, 35 slices, GRAPPA acceleration factor = 2 volumes, phase encoding direction A>P.

During resting state fMRI scans participants were instructed to stay still and keep their eyes closed without falling asleep, whilst receiving real and sham placebo brain stimulation – simultaneous tDCS-fMRI will be described in more details in Chapter III, Section 4.3.

3.5.5 DTI parameters

Diffusion-weighted volumes were acquired using a 64-direction protocol echoplanar imaging (EPI) sequence.

The following parameters were used: 2mm^3 isotropic voxel, distance factor 0%, interleaved order of acquisition, axial orientation, TR 9500ms, TE 103ms, field of view 256x256mm, 64 slices, EPI factor 128, GRAPPA acceleration factor = 2 volumes, phase encoding direction A>P, b-value 1 = 0s/mm^2, b-value 2 = 1000s/mm^2.

Standard (T1, T2 and fMRI) and advanced (DTI) phase map shimming was run before each sequence to measure and improve the magnetic field inhomogeneity. At the end of the exam, structural MRIs were sent for review to an expert consultant Neuroradiologist at the Hammersmith Hospital. In two cases, incidental findings (a frontal meningioma and grey matter loss) were reported, and participants were booked in to be seen in the appropriate clinic. The case of frontal meningioma was excluded from any analysis.

In the next section, the steps carried out to preprocess anatomical images, with the ultimate aim of linking pathological behaviour following a brain injury to the brain architecture, will be described.

3.6 Preprocessing of structural scans

The pipeline used to preprocess anatomical brain images is described in Sections 3.6.1-3.6.3 and summarised in Figure 2.5.

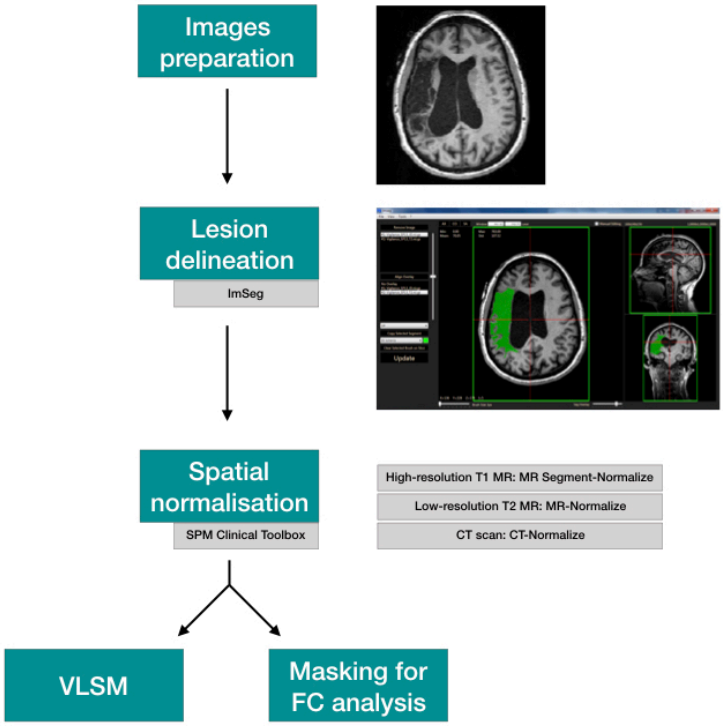


Figure 2.5: Pipeline for anatomical data pre-processing

3.6.1 Images preparation

Following brain scan acquisition, lesions had to be delineated on each slice of the patient's brain image. Out of the 26 stroke patients who took part in the studies described in this thesis, 22 were scanned at the CIF as part of the imaging study described in Chapter VI. Their high-definition MP RAGE scans were available on the CIF XNAT, an open-source imaging software platform dedicated to imaging-based research. For the remaining 4 patients who did not undergo a research scan, a CT/MRI scan was acquired at the time of each patient's admission on the 1.5T scanner from Siemens Healthcare at Charing Cross Hospital as part of standard clinical care (for imaging work with similar data, see Li et al., 2018; Rinne et al., 2018). These images were then obtained via the clinical PACS (Picture Archive and Communications System). All images, available in DICOM format, were then converted into NIFTI format using MRIcroGL. Volumes were then re-oriented along a conventional reference plane, the Montreal Neurological Institute (MNI) space defined by Talairach and Tournoux (Talairach & Tournoux, 1988), using FSL tool fslreorient2std - which flips axes preserving the quality of the original data.

3.6.2 Lesion delineation

Lesion maps were created on two machines running Windows 7 (HP EliteBook 2740p and HP Z800 Workstation) using the ImSeg Interactive Image Segmentation Tool (v1.8), developed by Dr Ben Glocker at Imperial College London. ImSeg is a semi-automated lesion delineation method that combines fully automated steps with user interactions: after specifying what represents healthy and what is damaged tissues on a few slices, the software semi-automates the process of drawing the lesion boundaries in other slices by computing an estimate of the lesion in 3D space – critically, this estimate has to be visually inspected and carefully refined manually on every slice. This software, which has been previously used to map pathologies following brain injury (e.g., see Li et al., 2019), was adopted because it allows delineating maps onto native space whilst overlaying an image acquired at a different time-point, or another weighted MRI image (e.g., T2, Diffusion Weighted Images (DWI) and fluid-attenuated inversion recovery (FLAIR) imaging) for best inspection of the lesion boundaries, ultimately improving the quality of the maps.

The following steps were performed to create lesion maps. For 22 out of 26 patients, MP RAGE slices 1mm thick constituted the main input and were loaded into ImSeg following an approach similar to that described by Mort and co-workers (Mort et al., 2003). For those patients who did not take part in the imaging study (n=4), lesion maps were traced onto native scans acquired for diagnostic purposes (a CT scan for one patient, a T2 scan for three patients). Once an input was loaded, the most appropriate modality was overlaid and aligned to achieve best precision - typically a T2 scan for research participants and FLAIR/SWI images for scans acquired for clinical purposes. The best possible combination of images was selected on an individual basis, i.e., the combination allowing optimal visualisation of the lesion responsible for the clinical presentation. In addition, to assist with lesion identification in cases where the boundaries were not obvious, diagnostic DWI and FLAIR imaging obtained by the clinical stroke services at Charing Cross Hospital were inspected in parallel.

Once the lesion was identified, it was demarcated in axial view on between 3 and 5 slices, which provided sufficient information for the algorithm to generate an estimate of the lesioned area. It was always necessary to subsequently check through the slices in full-manual mode, to ensure the boundaries were correct and refine the lesion map at the slice level. The same principles were applied consistently across the whole group of patients, and the resulting maps were re-inspected with other members of the research team and a Consultant Neurologist, to ensure no errors were made. Lesion masks were saved as binary maps.

3.6.3 Spatial normalisation

Before performing group analysis and comparing the extent of brain damage or brain activity between individuals, binary lesion maps reflecting the impaired voxel in each patient were normalised to a common stereotaxic space. The normalisation process warps the orientation, size and shape of an individual brain scan to match a template in MNI space (voxel size 1x1x1 mm). This process was performed using the spatial normalisation routines of SPM v12, as implemented in the Clinical Toolbox (Rorden, Bonilha, Fridriksson, Bender, & Karnath, 2012). In agreement with the preprocessing pipeline proposed by Karnath and co-workers (Karnath, Sperber, Wiesen, & de Haan, 2019), decisions were carefully made respecting the type of imaging data available in each case. Specifically, with low-resolution images (T2 MR for 3/27 and CT scan for 1/27), a traditional normalisation procedure was used; the template image was selected to match the image modality of the patient input (CT or T2), as the accuracy of the approach depends on the match between the two. For the vast majority of patients (22/26 patients), high-resolution T1 MR images were available and the unified segmentation-normalization approach (Ashburner & Friston, 2005) was chosen, as this has been shown to be robust for normalising scans with brain lesions (Andersen, Rapcsak, & Beeson, 2010; Crinion et al., 2007). The lesion was smoothed with a Gaussian kernel with a full-width half maximum (FWHM) at 3mm and a 0.5 threshold to remove jagged edges associated with manual drawing. Enantiomorphic normalisation was then performed (see Section 3.6.3.1 below,

'Correcting for the lesion') using the available MNI152 template of young participants (mean age=25, median age=24, standard deviation=4.9). Considering that one aim was to compare functional connectivity across the lifespan and in stroke, this would provide optimal correspondence between my imaging data in stroke and healthy younger and older adults. Also, it would enable the comparison of the data acquired as part of the study described in Chapter VI with other datasets from the imaging and stroke literature, mostly available in younger populations.

3.6.3.1 Correcting for the lesion

Normalization procedures are liable to distortions when there is an area of brain damage, especially when non-linear warping is used (Andersen et al., 2010; Brett, Leff, Rorden, & Ashburner, 2001; Nachev, Coulthard, Jager, Kennard, & Husain, 2008). Two dominant methods to mitigate the effect of a lesion have been described, and they are both available in the Clinical Toolbox in SPM. In lesion cost function masking, regions identified as abnormal do not contribute to the normalization transforms (Brett et al., 2001). In other words, the necessary transformations are derived from intact areas only, making this process less accurate as the lesion size increases. During enantiomorphic normalization, regions of brain damage are replaced with tissue from the healthy hemisphere's homologue area (Nachev et al., 2008). The transformation in this case are effectively derived from a brain without a lesion, which makes this method more suitable in cases of large unilateral lesions (Rorden et al., 2012).
For the purpose of this work, the decision to use either function masking or enantiomorphic normalisation was made on an individual, patient-to-patient basis as recommended by Karnath and colleagues (Karnath, Sperber, Wiesen, et al., 2019), depending on the available input (e.g., enantiomorphic necessarily requires a high-definition T1) and by comparing the outcome of each method. Visual examination of the renderings allowed to detect poor normalisation, as systematic distortions were clearly visible (Rorden et al., 2012). The normalisation process created a normalized bias corrected anatomical image and a binarised, warped, smoothed lesion map. I carefully inspected the fit between these and the template

using the 'check registration' tool in SPM, and by overlapping them using MRIcron software package (Rorden & Brett, 2000) (https://people.cas.sc.edu/rorden/mricron/index.html). When no clear preference for any of the two methods existed, I chose enantiomorphic as recommended by Karnath and colleagues (Karnath, Sperber, Wiesen, et al., 2019). A satisfactory quality of the normalised map was achieved in all cases.

Figure 2.6 shows the overlap of lesion maps for all 26 patients, created by superimposing all maps to the CH2 template in MRIcron. The majority of patients that took part in the studies described in this thesis had damage within the territory of the right middle cerebral artery (MCA).

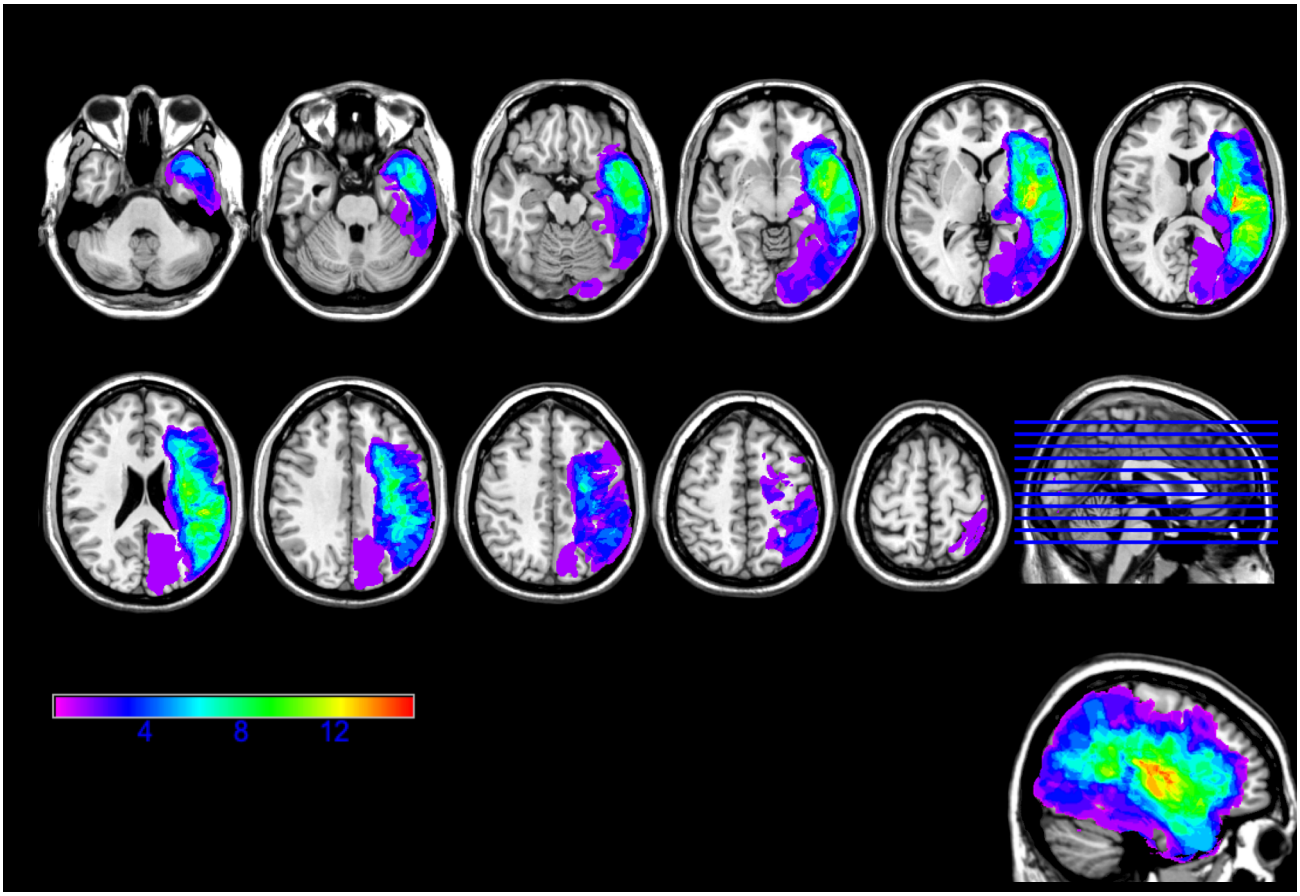


Figure 2.6: Overlay lesion plot for 26 right-hemispheric stroke patients with neglect
Shown are axial views, a sagittal image with slice levels generated with MRIcron and a lesion rendering of the lesion distribution for all patients. Colour areas depict the extension of the lesion, with shades of blue proportionally getting lighter as the degree of overlapping among lesions between subjects increases.
The number of patients with damage to each region is represented by the multi-coloured bar, which shows the number of individuals with damage at each voxel: one patient depicted by the most violet colour, and up to 14 patients overlapping as indicated by red-yellow colour in the parietal region.

The resulting maps could then be used for further analysis: they were used to compare lesion anatomy with task performance and response to treatment, and to mask the lesion for functional imaging analysis (Chapter VI). The rationale, together with limitations associated with lesion-symptom mapping, are appraised in the next section.

3.7 Lesion-behaviour mapping

In-vivo visualisation of the human brain has transformed the understanding of brain-behaviour relations. The so-called 'lesion method' allows linking areas of identified focal brain damage with a clinical manifestation (Rorden & Karnath, 2004). Voxel-based lesion-symptom mapping (VLSM) (Bates et al., 2003; Rorden, Karnath, & Bonilha, 2007) is a powerful approach in furthering our understanding of the underpinning of behaviour, as it can reveal a direct relationship between structure and function (Karnath, Sperber, & Rorden, 2019). This method applies an independent statistical test at a voxel level to relate voxel status (lesioned or not lesioned) with the behaviour of interest, and generate a voxel-wise map of statistical significance (Bates et al., 2003). Specifically to this thesis, this method was applied to reveal relationships between anatomy and task performance, and between anatomy and response to a treatment that involved the application of brain stimulation (see Chapter VI, Section 2). The use of VLSM has its strength in the fact that it is understood to reveal if a certain region is necessary rather than merely involved with a behaviour (for a review, see Rorden & Karnath, 2004). However, there are a number of issues related to its use. Some of them are discussed below.

3.7.1 Images acquisition

The identification of a lesion location and size necessarily depends on the methods used for acquiring the images. For clinical purposes, CT and T2/FLAIR MRI brain scans are normally acquired to diagnose and monitor a stroke. However, the use of clinical CT/MRI scans to map lesions appears to be associated with a number of confounds (Malhotra & Russell, 2015).

In the present work, a high-definition protocol (1mm thick slices) was used to obtain detailed anatomical information for 85% of the patients. For the remaining patients, clinical scans only were available. Although the resolution was far lower, from a technical point of view there was no need to exclude this subset of patients: the clinical toolbox utilised for normalisation purposes provides templates that

allow spatial normalisation of both CT and MR scans, and is ideally suited for clinical studies where different imaging modalities are present in different patients. It would have been a loss of representativeness, if those cases had to be excluded from this study due to incompatibility with the scanning procedure.

3.7.2 Mapping accuracy

The rationale behind the VLSM approach is that the lesion responsible for the symptoms is to be mapped. Small vessel disease, seen as white matter hyperintensities on scan, and general brain atrophy, seen as enlarged sulci which also affect general brain functioning, are not identified, even if they most certainly contribute to the brain general susceptibility at the time of the event (Pendlebury & Rothwell, 2019). This consideration applies to the present work as well as VLSM studies as a whole: lesions that were considered responsible for the current presentation were mapped, whereas small vessel disease was not included, although its exact contribution to cognitive deficits has not been fully explored. The very same approach and strategy were used consistently and systematically by me to process structural imaging.

For lesion delineation, a first semi-automatic step for lesion delineation was used. It was noticed that lesions close to the ventricles, the inter-hemispheric fissure, or to the scalp were particularly problematic when using semi-automated methods (e.g., Stamatakis & Tyler, 2005). To mitigate this problem, careful refining was always performed in full manual mode, at the slice level.

3.7.3 Staging of brain pathology

Studies may vary as regards to the timing when scanning was performed. In the acute stage, symptoms may be related to impaired functions in nearby regions (Malhotra & Russell, 2015). Also, there is often acute oedema, and the lesion appears larger in size. These factors, together with the important consideration that many acute patients would not be able to tolerate a session in the scanner,

influenced the decision, for the purpose of this work, to scan patients in the chronic stage of illness (> 3 months post-stroke).

It should be noted that the process of delineating the lesion was found to be particularly difficult when the scan was performed years after the acute event. By then, encephalomalacia is sometimes difficult to differentiate precisely from enlarged ventricles and sulci relating to atrophy, making it harder to see the boundaries of the pathology. This issue was partially overcome by overlapping a modality acquired in acute stages to assist with the drawing – this option is available within the software ImSeg.

3.7.4 White matter

Another issue with this general approach is the bias towards recognising cortical areas, with poor sensitivity for detecting injury along white matter tracts (Malhotra & Russell, 2015). Stroke anatomy is however increasingly recognised to have key subcortical components (Corbetta et al., 2015), and brain damage may extend well beyond the area of apparent grey matter injury, with disconnections leading to remote dysfunction of apparently intact cortical lesion (Bonilha, Rorden, & Fridriksson, 2014; Carrera & Tononi, 2014; Catani & Mesulam, 2008; Fridriksson, Bonilha, & Rorden, 2007; Mukherjee, 2005).

In the present work, a broad exploratory approach was taken, with the aim of identifying both cortical and subcortical areas that were linked to task performance or necessary for any tDCS effect to occur. Specifically, it was considered a worthwhile approach to use a brain atlas that contained maps for both cortical and subcortical areas.

4 Conclusions

The set of behavioural assessments and the imaging protocol detailed above were optimised to examine individuals affected by attentional difficulties following right-hemisphere stroke. These methods were considered to be best suited for the purpose of the work described in this thesis.

Indeed, clinical testing was feasible and pragmatic enough to allow testing of the majority of patients on acute wards, including the sickest patients cared for in the time-pressured NHS environment. It was able to capture attentional difficulties in a heterogeneous clinical group of patients which can be considered to be representative of the population of individuals affected by neglect.

The imaging protocol, optimised for capturing brain anatomy and functioning, was created to maximise tolerability during data acquisition in patients with different stroke severity. The choice of a resting state fMRI protocol, with minimal demands on participants, for instance, contributed to make the scanning session accessible to the most severe patients, who are normally excluded from imaging research.

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Chapter III: Brain Stimulation and Functional Imaging Methods

1 Introduction

In this chapter, transcranial Direct Current Stimulation (tDCS) methodology in the context of stroke will be discussed. Recent developments that make the delivery of tDCS possible in an MRI environment will also be detailed, together with the pre-processing steps performed on imaging data in preparation for resting-state functional connectivity analysis.

2 Transcranial direct current stimulation

The field of electrical brain stimulation is expanding rapidly - from fewer than 30 papers published in 2004 to over 750 in 2019 – an increase of over 2000% in 15 years (PubMed search, 'transcranial direct current stimulation'). TDCS is a technique that allows modulating spontaneous brain activity non-invasively. As opposed to transcranial magnetic stimulation (TMS), which affects brain activity by directly depolarising the soma and initiating action potentials (Klomjai, Katz, & Lackmy-Vallee, 2015), tDCS exerts its effects by promoting changes in cells' resting membrane potential. The application of a weak direct electrical current alters spontaneous firing rate and renders neurons more or less prone to respond, ultimately shaping synaptic efficacy (Miniussi, Harris, & Ruzzoli, 2013).

Elements of the brain stimulation methods described in this chapter have been published in a special issue on neglect. Olgiati, E., & Malhotra, P. (2020). Using non-invasive transcranial Direct Current Stimulation for Neglect and Associated Attentional Deficits following Stroke. Neuropsychol Rehabil.
Elements of the targeted tDCS montage described in this chapter have been published in abstract form. Olgiati, E., Violante, I.R., Li, L.M., Faraj, A., Sinclair, T., Crow, J., Wise, R., & Malhotra, P. (2019). Targeted brain stimulation to ameliorate vigilance in stroke: a combined tDCS-fMRI approach. Brain Stimulation 12(2):573.

Given its potential to promote brain plasticity, tDCS may have a role in fostering brain circuitry reorganisation after a stroke (Vallar & Bolognini, 2011).

The process of deciding on the most appropriate design for a tDCS study is a multifaceted process that requires iterative considerations before the most suitable (and feasible, given available resources) configuration is chosen (Jaberzadeh, Martin, Knotkova, & Woods, 2019). Considering that there are no specific guidelines for clinical tDCS studies (see consensus paper by Brunoni et al., 2012), I discuss below a framework of critical parameters that nevertheless require careful consideration when designing and evaluating a treatment involving the application of tDCS in stroke patients.
This framework has been established to design the tDCS protocol for the studies described in this thesis. As such, it is particularly suited to patient studies, specifically patients with a brain injury. It has therefore been developed to evaluate the potential therapeutic effect of stimulation rather than using tDCS as methodology to understand brain function.

2.1 tDCS safety in stroke

Paramount in any study assessing a therapeutic intervention is the need to ensure patient safety. Current safety recommendations for non-invasive brain stimulation include avoiding the use of extra-cephalic montages (i.e., where the reference electrode is placed over the deltoid muscle or the neck) in frail populations such as the elderly and individuals with comorbidities. This is because of the associated risk of shunting of electric current through the skin and the risk of influencing brainstem activity (List et al., 2015; Vernieri et al., 2010).
Correspondingly, in the present studies, an intra-cephalic tDCS setup was implemented, with electrodes positioned solely over the head. For a recent review of safety recommendation for tDCS in stroke, also see Russo and co-workers (Russo, Carneiro, Bolognini, & Fregni, 2017).

2.2 tDCS in cases of extensive brain damage

Some studies have suggested that patients with larger lesion volume (Bolognini et al., 2014), white matter tract disconnection (Bradnam, Stinear, & Byblow, 2013; Li, Violante, Zimmerman, et al., 2019; Lindenberg, Nachtigall, Meinzer, Sieg, & Flöel, 2013; Rosso et al., 2014) or more severe impairments (Bradnam, Stinear, Barber, & Byblow, 2012; Marquez, van Vliet, McElduff, Lagopoulos, & Parsons, 2015; O'Shea et al., 2014), may benefit to a lesser extent from the application of tDCS.
In these scenarios, electrode location appears to be critical, particularly in cases of extensive brain damage following stroke which is more likely be associated with all of the above. This is because electric current will always follow the path of least resistance, and is likely to dissipate through the cerebrospinal fluid (CSF) that occupies the lesion space as encephalomalacia develops (Fernandez-Corazza, Turovets, Luu, Anderson, & Tucker, 2016). Targeting surviving brain tissue and thus ensuring that the current is travelling outside the lesioned area seems therefore essential if stimulation is to have any therapeutic effect. In an effort to avoid stimulating lesioned tissue, electrodes can be positioned above spared regions of the ipsilesional hemisphere or the homologue area in the contralesional hemisphere. The evidence available to date suggests that targeting the lesioned hemisphere may be more effective (Stagg et al., 2012), with a reported detrimental effect of tDCS inhibiting activity of the contralesional hemisphere in severely affected patients (Johansen-Berg et al., 2002).
In the present study, a precise targeting of spared regions within the affected (right) hemisphere was aimed for by using smaller electrodes clustered around the desired area. Lesion volume and location were assessed in order to examine whether they were linked to tDCS responsiveness, in an effort to identify potential predictors of response to stimulation.

2.3 Timing of stimulation

Another aspect to be taken into consideration when designing a clinical study regards the optimal timing of treatment in relation to stroke onset. At present, there is very little evidence suggesting a clearly defined optimal therapeutic

window to administer tDCS. Most studies in the literature have applied tDCS in the post-acute and chronic stages post-stroke. Interestingly, some studies exploring the potential of an early application of tDCS (either alone or administered during a rehabilitation session), showed that the degree of tDCS-related improvement was greater at follow-up rather than at treatment cessation, although it should be noted that these studies were examining effects in the motor domain only (Andrade et al., 2017; Bornheim, Croisier, Maquet, & Kaux, 2019; Sattler et al., 2015). A number of other studies, however, have not found evidence supporting a role for stimulation in acute stages post-stroke (Di Lazzaro et al., 2014; Rossi, Sallustio, Di Legge, Stanzione, & Koch, 2013).

The present investigation was conducted in a sample of chronic stroke patients. This strategy allows most of the spontaneous recovery to occur before applying tDCS: a stable baseline makes it easier to establish whether there is an actual treatment effect beyond spontaneous recovery. Indeed, patients affected by neglect are typically left with severe chronic attentional deficits that tend to last (see Chapter I, Section 2.5), and the aim of the present work was to explore a potential avenue to alleviate such persisting impairments.

2.4 tDCS sessions

There is no standard definition of tDCS dose in clinical studies. The dose-response relationship for tDCS appears to be complex and non-linear in healthy individuals (Jamil et al., 2017), and has not been systematically investigated in clinical populations. Typical dosage in clinical studies involves 10-20 minutes of 0.5-2mA tDCS delivered via two large electrodes (Brunoni et al., 2012).

Studies also vary as to how many doses of tDCS are administered. In a common tDCS design, a single dose of stimulation is applied and any change in behavioural measures is recorded and compared to a sham group/session. Physiologically, a single dose is considered to induce short-term acute effects on cortical excitability, with after-effects lasting for about an hour (Nitsche et al., 2003). In multiple-dose approaches, the effect of the repeated application of tDCS, applied once a day for several days, are evaluated. There is evidence showing accumulation of

modulatory effects following the repeated application of tDCS over consecutive sessions (Alonzo, Brassil, Taylor, Martin, & Loo, 2012; Reis et al., 2009; Waters-Metenier, Husain, Wiestler, & Diedrichsen, 2014), with long-lasting LTP-like effects persisting beyond 24 hours in healthy humans (Monte-Silva et al., 2013).
The studies described in this thesis are proof-of-principle studies that examined the immediate effect of one application of tDCS on behaviour and brain networks. This choice was intended to explore the feasibility and tolerability of the tDCS approach and explore if it had any therapeutic potential. If there was a suggestion of a beneficial effect of this approach, it would be appropriate to explore this further using a randomised clinical trial design incorporating multiple doses.

2.5 Online vs Offline tDCS

Recent studies have highlighted that the timing of stimulation in relation to a function of interest is a key variable in determining the effect of tDCS (Pirulli, Fertonani, & Miniussi, 2013, 2014; Stagg et al., 2011; Stagg & Nitsche, 2011). From a methodological perspective, tDCS studies can be distinguished between online and offline paradigms, depending on whether any activity is carried out whilst receiving brain stimulation. In this context, online refers to those paradigms where tDCS is applied during a behavioural task or training, whereas offline indicates paradigms where tDCS is applied at rest, prior to commencing a task or training. It is possible that if a task is performed whilst receiving stimulation (online), tDCS may act on the networks and brain regions which are most active during brain stimulation - meaning that the choice of the task performed during stimulation is likely to be key in determining its effect (Gill, Shah-Basak, & Hamilton, 2015). However, it should also be noted that in cases where tDCS is applied at rest, any task performed immediately before stimulation may also have affected and primed brain networks (Bijsterbosch, Smith, & Beckmann, 2017) - this information has not been always reported in studies utilising offline paradigms, but it is potentially relevant for interpreting conflicting evidence in the literature.
In two studies described in this thesis, tDCS was delivered online, i.e. during task performance (Chapter IV and V). In another study, participants performed an

attentional task before entering the scanner, where they received tDCS whilst undergoing fMRI scanning (Chapter VI).

2.6 Stimulation polarity and directionality

The canonical assumption in the tDCS literature is that anodal stimulation causes a physiological increase in cortical excitability, with the intended behavioural effect of upregulating activity in the stimulated area; conversely cathodal stimulation is thought to decrease cortical excitability, inhibiting brain activity (Nitsche & Paulus, 2000). However, this relatively simplistic dual-polarity model has recently been reconsidered, with reported outcomes in the opposite direction. For instance, in the motor domain, it has been found that only about 36% of participants showed the standard pattern of anodal stimulation producing facilitation and cathodal stimulation causing inhibition (Wiethoff, Hamada, & Rothwell, 2014). Results from studies examining effects on regions outside motor cortex appear to be even more complex and conflicting (Brückner & Kammer, 2017; Jacobson, Koslowsky, & Lavidor, 2012; Li, Violante, Leech, Ross, et al., 2019).

An alternative approach to focussing on the effects of electrode polarity at a single electrode location is to focus on stimulation directionality: as the current leaves the anode and passes through the cortex while travelling to the cathode, it affects neuronal resting-state potentials. There is now evidence showing that the current does not only affect the area directly beneath or near the electrodes, but has a more widespread effect including relatively distant areas (Lang et al., 2005). By using ex-vivo modelling and simulations of the current flow based on realistic head models, it may be possible to develop an increased understanding of the most likely effects achievable with different electrode configurations (Bikson, Rahman, & Datta, 2012).

Different set-ups were considered when deciding on a tDCS montage for the present study. The chosen configuration allowed the optimal targeting of a cortical area, which was confirmed by computational modelling of the current distribution.

2.7 State-dependent tDCS

In an effort to increase our understanding of the factors behind the reported large interindividual variability in tDCS outcomes (Wiethoff et al., 2014), studies in healthy volunteers have researched how the state of the brain at tDCS delivery may influence response to tDCS (Li, Uehara, & Hanakawa, 2015). In addition to the task performed during/prior to stimulation (see Section 2.6 'Online vs Offline tDCS'), other potential state-related factors include baseline task sensitivity (Benwell, Learmonth, Miniussi, Harvey, & Thut, 2015; Learmonth, Thut, Benwell, & Harvey, 2015; Tseng et al., 2012) and baseline network activity (Antal, Terney, Poreisz, & Paulus, 2007; Li, Violante, Leech, Ross, et al., 2019). This line of investigation in healthy individuals highlights the importance of considering a behavioural and network baseline on which to map the effect of tDCS and potentially identify responders and non-responders in clinical populations. In the present thesis, I have not directly explored these issues but have instead focussed on the effects of age and lesion anatomy on tDCS responsiveness. Both these factors, particularly the latter, are likely to have a profound effect on responsiveness in the context of patients with attentional deficits following stroke.

2.8 Stimulation focality

In conventional tDCS, current is typically applied to the scalp via two large (typically 5x7cm or 5x5cm) saline-soaked sponge electrodes kept in position using non-conductive elastic straps. They may be positioned above the same hemisphere (as in single-mode tDCS) or above regions in both hemispheres (as in dual-mode or oppositional tDCS, when the reference electrode targets the contralateral homologue area). The spatial resolution achievable with such montages is low, with current spreading over several gyri (Nitsche et al., 2007). Thus, such a conventional tDCS setup with bipolar large electrodes, is thought to activate the region of interest as well as collateral cortical and subcortical surrounding tissue (Moreno-Duarte et al., 2014). Computational studies have indeed confirmed wide electrical field distributions, with maximum field strengths not located directly

underneath the electrodes but at an intermediate location outside the desired target region (Datta et al., 2009; Klaus & Schutter, 2018).

There is growing interest in moving on from the use of two large electrode pads in favour of High Definition (HD) tDCS montages featuring smaller disc electrodes, in order to increase precision and focality to a desired target (Datta et al., 2009; Dmochowski, Datta, Bikson, Su, & Parra, 2011; Dmochowski et al., 2013; Dmochowski, Koessler, Norcia, Bikson, & Parra, 2017; Guler et al., 2016; Ruffini, Fox, Ripolles, Miranda, & Pascual-Leone, 2014; Sadleir, Vannorsdall, Schretlen, & Gordon, 2012; Saturnino, Madsen, Siebner, & Thielscher, 2017; Wagner et al., 2016). Local targeting has been achieved using varied electrode configurations, typically one source electrode surrounded by two or more sinks and concentric-ring configurations; in such HD-tDCS setups, an increase in spatial resolution is obtained using small electrodes arranged in close proximity (Kuo et al., 2013). This leads to a substantial shunting of the current through the scalp, so that the current does not reach deeper brain regions as it would with distant large pads (Huang & Parra, 2019). Denser electrode arrays of HD-tDCS therefore seem ideal when trying to confine the area of stimulation and limit the current in the spatially constrained superficial area between the electrodes.

Considering that individuals with neglect often have large lesions, in the current studies I used HD-tDCS rather than a more conventional stimulation approach: this allowed maximum focality and precise targeting of spared regions, whilst avoiding current dissipation through the cerebrospinal fluid that fills the space once occupied by the lesion. In healthy individuals, the two techniques seem to induce comparable changes in cortical plasticity and have similar tolerability profiles (Kuo et al., 2013), with similar behavioural effects on an attentional task (Hogeveen et al., 2016).

2.9 tDCS blinding

One of the main advantages of tDCS as compared to other forms of neurostimulation is the standard placebo condition, assumed to be undistinguishable from the active condition: the current is ramped-up, plateaued and then ramped-down in a few seconds. This produces sensations on the skin comparable to active stimulation, but without neuromodulatory effects (Nitsche et al., 2008). There are however some reports suggesting that blinding can be difficult to achieve with intensities of 2mA (O'Connell et al., 2012) and even 1mA (Greinacher, Buhôt, Möller, & Learmonth, 2019; Turi et al., 2019) in younger healthy adults. In older and clinical populations, blinding effectiveness has not been systematically addressed to date; available evidence suggests successful participants blinding (Gandiga, Hummel, & Cohen, 2006). Only a minority of studies have however utilised HD-tDCS in these populations (e.g., see Datta et al., 2009; Richardson, Datta, Dmochowski, Parra, & Fridriksson, 2015).
Given the relative paucity of previous studies using this technique in older and clinical populations, I used the same stimulation approach, involving the delivery of targeted tDCS, when testing healthy individuals in all age groups and also in stroke patients. Any cutaneous sensations and potential side-effects associated with tDCS were documented for all participants. The tolerability profile of the technique, both alone and in combination with fMRI, was therefore systematically assessed.

3 tDCS Methods

One of the key aims of the present investigation was to examine the effects of tDCS, across the life span and following right-hemispheric stroke, using a precise targeting of the right-lateralised frontoparietal vigilant attention network (Olgiati et al., 2019). Damage to this network has repeatedly demonstrated to be critical in the pathophysiology of non-lateralised attentional deficits in right hemisphere stroke, and in the pathogenesis of neglect (Corbetta & Shulman, 2011).

Following on previous work in healthy individuals (Brosnan et al., 2018; McIntire, McKinley, Nelson, & Goodyear, 2017; Nelson, McKinley, Golob, Warm, & Parasuraman, 2014), a decision was made to target the right dorsolateral prefrontal cortex (DLPFC), which is typically spared in neglect patients. Previous studies that aimed to modulate activity of the right DLPFC positioned the anodal electrode over F4 as determined by the EEG 10-20 international system (e.g., see Brosnan et al., 2018). However, the DLPFC lies just beneath this area. This may not be particularly relevant when using conventional large electrodes, but it is critical for when the current is confined in a smaller region as in HD-tDCS approaches. Different montages were therefore modelled, until optimal and precise targeting of the right DLPFC was achieved.

3.1 Exploratory electric field visualisation

Given the difficulty of obtaining in-vivo measurements of current density, computational forward models of conventional and alternative electrode montages can be used to provide accurate insight into current flow patterns and tDCS montage performance (Bikson et al., 2012; Noetscher, Yanamadala, Makarov, & Pascual-Leone, 2014). These are typically applied to predict current flow and characterise the impact of surface electrodes arrangement, with the ultimate aim of achieving optimal targeting or avoidance of given brain regions.

In the context of this research study, in order to explore the distribution of the current originated by different electrode configurations, I initially simulated the tDCS electric fields using StimViewer, a software component embedded in the Neuroelectrics Instrument Controller (https://www.neuroelectrics.com/products/software/nic2/). This is a simulation engine which reproduces the electric fields associated with a given montage and displays them with respect to grey matter using a realistic head model as defined by Miranda and co-workers (Miranda, Mekonnen, Salvador, & Ruffini, 2013). Briefly, tissue boundaries are derived from MR images and the Finite Element Method (FEM) is used to calculate the electric potential in the head (for more

details about principles and assumptions of this model, see wiki.neuroelectrics.com). Following the International 10-20 system, different montages were simulated. The electrode arrangement that allowed most precise targeting of the right DLPFC consisted of a triangular montage with three electrodes (one source and two sinks), positioned over F4 (anode), F8 and FP2 (cathodes) (Figure 3.1). This montage was subsequently used in all the studies for this thesis.

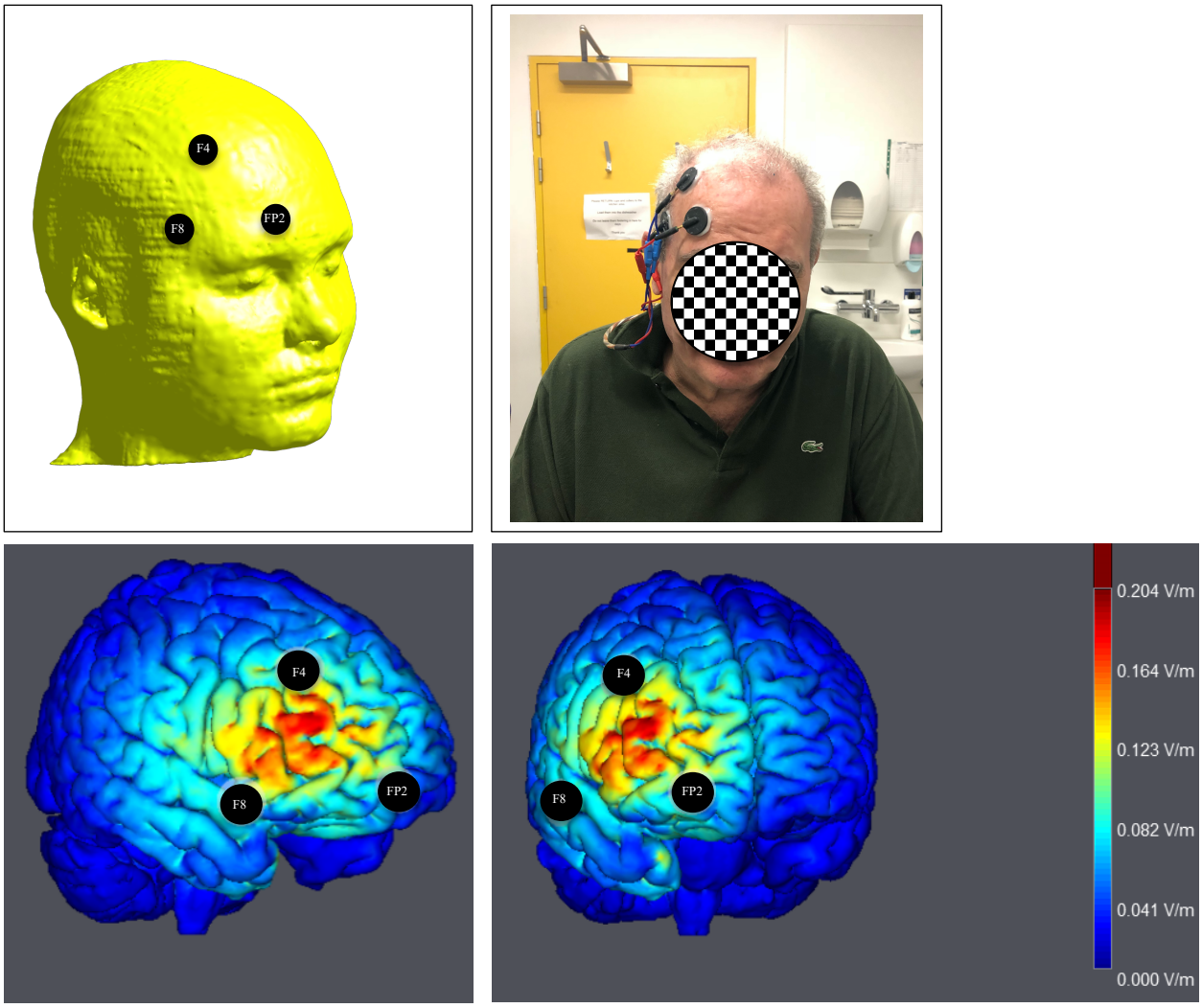


Figure 3.1: tDCS montage for optimal targeting of the right dorsolateral prefrontal cortex (DLPFC)
Electrodes are arranged in a triangular configuration, with anodal electrode over F4 and returns over F8 and FP2. Upper panels: 3D head model and a study participant displaying the tDCS set up for the study. Lower panels: 3D model of the brain (in lateral and frontal view) showing the electric fields associated with this montage. Values are shown as electrical field total magnitude ||E|| measured in Volts/m.

3.2 Confirmatory electric field visualisation

To confirm the precise current field distribution and magnitude that I would achieve with the specific (MR-compatible) tDCS equipment that was available in the laboratory, a confirmatory electromagnetic FEM simulation was performed using the software Sim4Life from Zurich MedTech AG (https://zmt.swiss/sim4life/video-training/).

This model was set up using the following parameters:

- Three circular electrodes 1.5cm ⌀;
- 5 mm electrode thickness, considering electrode and gel layer;
- Electrodes location with anode over F4, cathodes over F8 and FP2 (International EEG 10-20 system).

The computational model performed a simulation of the electric field distribution and currents as well as the related impact on neuronal activity. The FEM modelling was created with a spatial resolution of 1x1x1mm, with anode delivering 1mA and cathodes attracting 0.50 each. This was possible by combining the field distributions from the montage with the MIDA head model, a multimodal ultra-resolution head and neck model (Iacono et al., 2015).

A potential limitation to this approach is that there are individual differences in anatomy; however, it is not possible yet to simulate through personalised models and the MIDA model represents the gold standard for computational modelling involving detailed representations of the human brain anatomy.

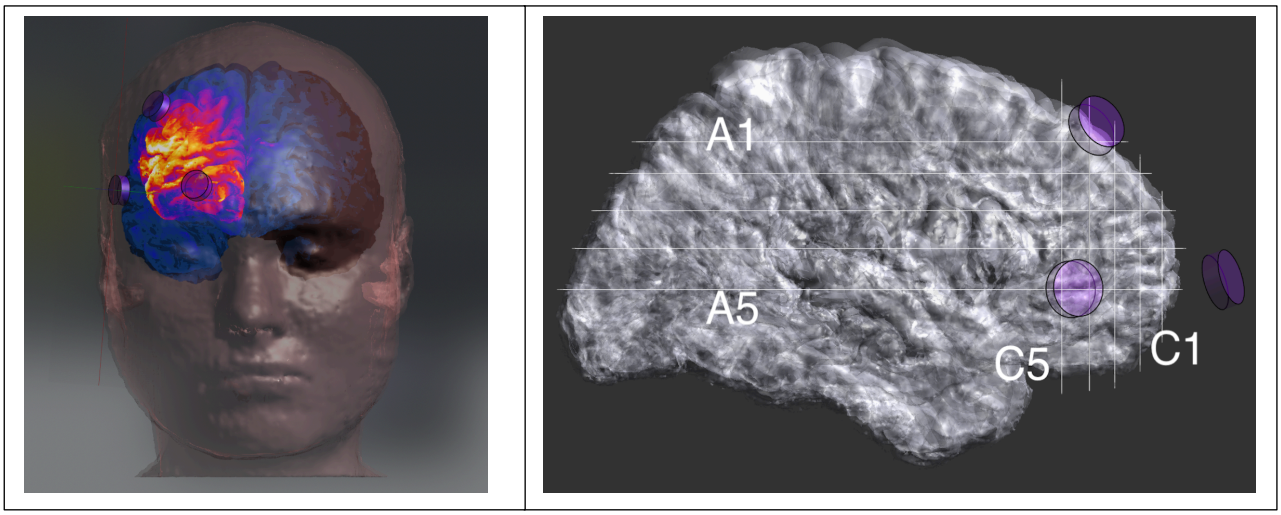


Figure 3.2: FEM modelling of the electrical field distribution, with grey matter superimposed (modelled using Sim4Life)
Left panel: Electrode configuration in coronal view. Right panel: Slices in axial plane (A1-A5) and coronal plane (C1-C5) that will be visualised.

The computation model confirmed that the peak electric field strength was over the right dorsolateral prefrontal cortex (DLPFC) with this triangular montage (Figures 3.2-3.4). Similar electromagnetic field computations are being now utilised more and more to simulate the distribution of the fields in neuromodulation studies employing tDCS (Datta, Zhou, Su, Parra, & Bikson, 2013; Laakso et al., 2016; Seibt, Truong, Khadka, Huang, & Bikson, 2019) or other stimulation techniques (e.g., see Grossman et al., 2017).

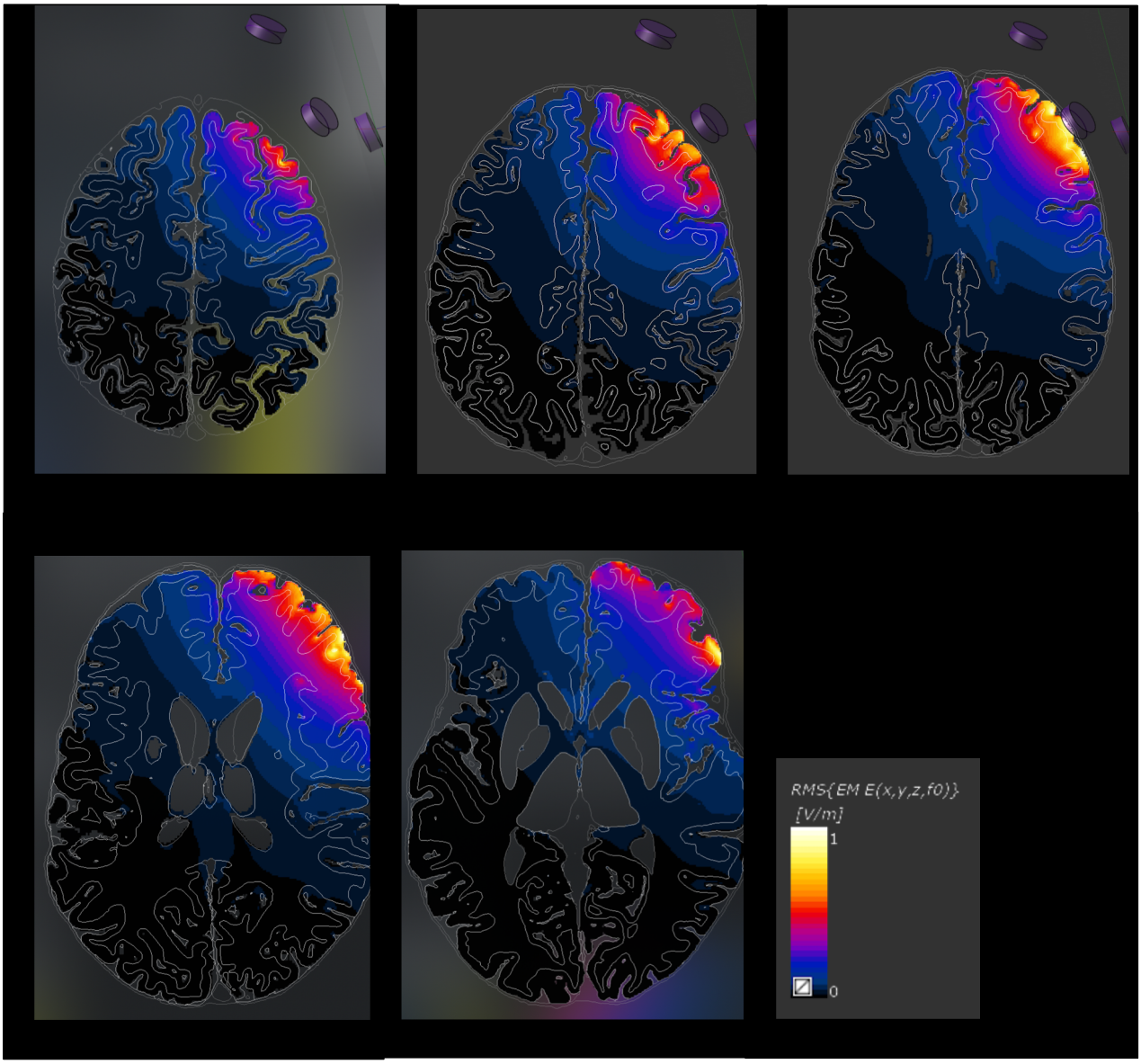


Figure 3.3: FEM modelling in axial plane of the electrical field distribution, with grey matter superimposed (modelled using Sim4Life)
The model shows the focality achievable with the tDCS montage used in the present work. Slices A1-A5 from Figure 3.2 are shown.

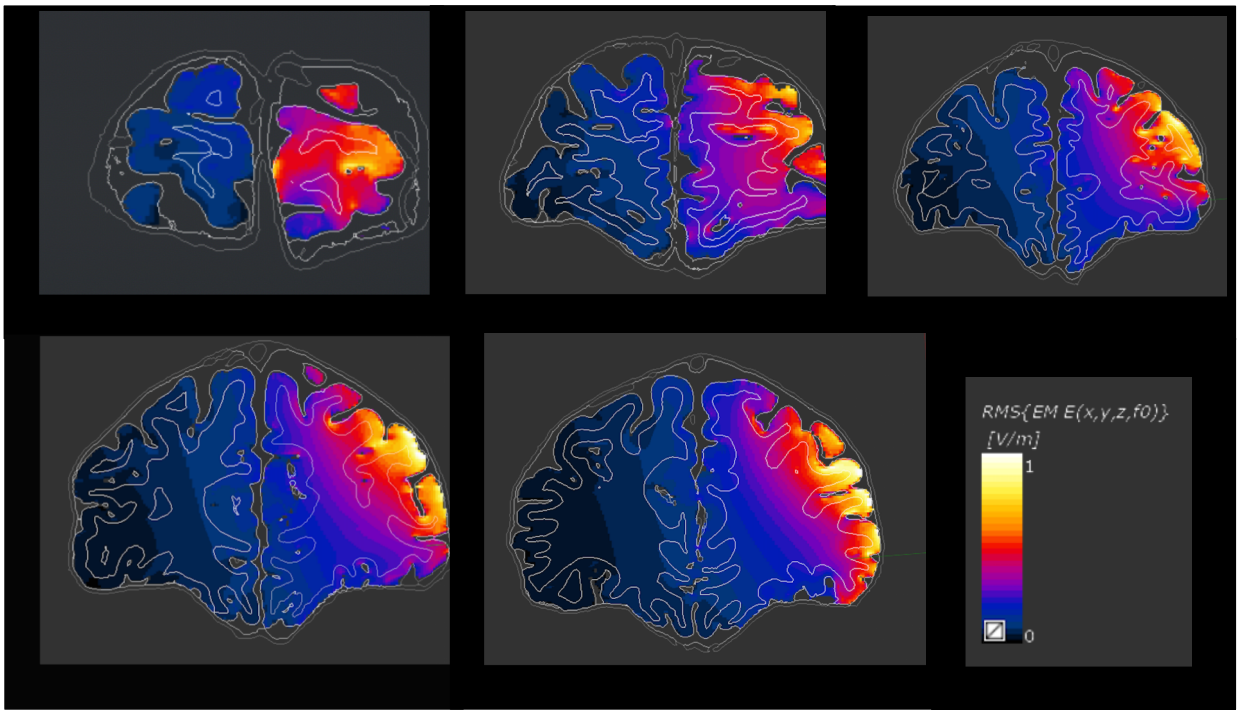


Figure 3.4: FEM modelling in coronal plane of the electrical field distribution, with grey matter superimposed (modelled using Sim4Life)
The model shows the depth of current penetration achievable with the tDCS montage used in the present work. Slices C1-C5 from Figure 3.2 are shown.

3.3 tDCS apparatus

The apparatus used for the delivery of tDCS is shown in Figure 3.5. Direct current stimulation was delivered using two sets of battery-powered stimulators (neuroConn GmbH, Ilmenau, Germany) connected to two filter boxes via two sets of MR-compatible wires. From the filter boxes, the signal reached three disc electrodes (15mm Ø) via custom-made MR-compatible wires, so that the anode of both stimulators was combined in one electrode. Stimulators were controlled through a digital to analogue converter (DAQ) (National Instruments, Newbury, UK) receiving output from in-house MATLAB scripts. The beginning of each stimulation session was controlled via an external trigger sent to the DAQ from a computer running a MATLAB script that specified the stimulation condition (real or sham) for a given participant, using a random order matrix set by another researcher, thus ensuring blinding of the experimenter.

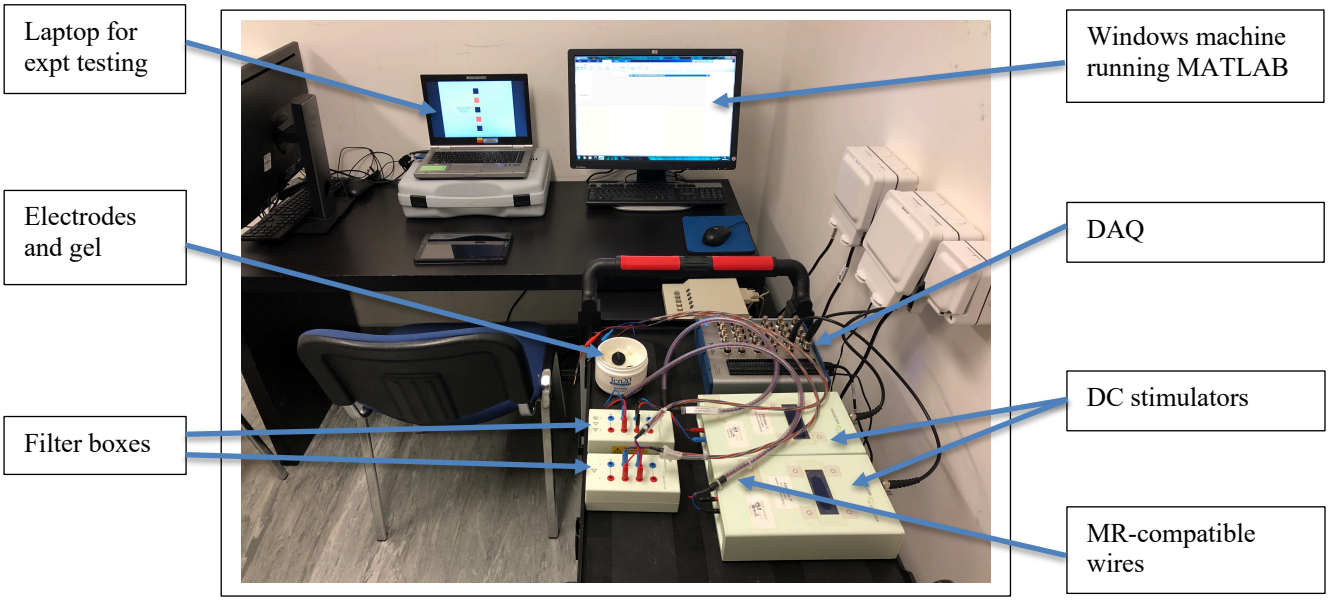


Figure 3.5: tDCS apparatus

3.4 tDCS set-up

Electrode location was determined by having each participant wear a flexible (size 56 or 58cm, as appropriate) EEG cap, with position sites labelled with a letter and a number according to the International 10/20 Electroencephalogram System (Figure 3.6).



Figure 3.6: Two participants wearing the EEG cap for head measurements

Four skull landmarks were measured: nasion (i.e., bridge of the nose), inion (i.e., external occipital protuberance) and two preauricular points (i.e., small portion of cartilage projecting anteriorly to the pinna). Measuring tape was applied along the midline, covering the distance from the inion to the nasion, and the position of the cap was adjusted so that the site for the vertex on the cap would be positioned halfway. The tape was reapplied transversally between the two preauricular points to find the location correctly aligned in both horizontal and vertical planes. A crayon was used to mark where the electrodes would go.

The cap was then removed, and the electrodes held in place on the head using a layer of TEN20 EEG conductive paste, which also kept impedances below 5kΩ. This is in keeping with other studies advising on the preferability of conductive paste to avoid the prolongation of preparation times and reduce the probability of resistance rise, leading to pain and burning sensations, and potentially study drop-out (Antonenko et al., 2017).

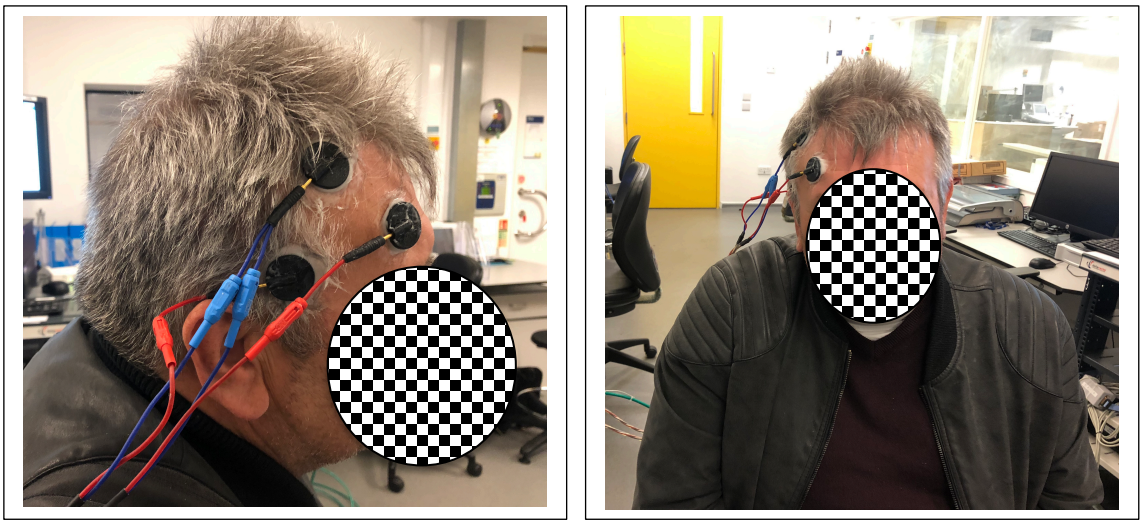


Figure 3.7: A study participant with electrodes on
Note the electrodes arranged in a triangular montage with anode (blue wire) and cathode (red wires).

4 Resting-state functional imaging

The brain is operational even during a relaxing state: it is estimated that 60-80% of the energy used by the brain serves to support the constant communication between cells; on the other hand, task-related increase in metabolism accounts for about 1% of the energy (Gusnard, Akbudak, Shulman, & Raichle, 2001; Raichle & Mintun, 2006). This continuous communication between cells is not random, as the brain appears to be highly organised into cortical network systems with specific functions and varied spatial topology (Smitha et al., 2017). When the brain is 'at rest', a large-scale inherent organisation has been consistently demonstrated with the use of resting state functional magnetic resonance imaging (R-fMRI), a cutting-edge technique for assessment of brain networks (Damoiseaux et al., 2006; Fox & Raichle, 2007). Such coherent patterns of spontaneous physiological fluctuations have been documented across different states such as sleep and anaesthesia, suggesting that resting state networks (RSN) represent an intrinsic property of the brain as opposed to unconstrained mental activity (Fox & Raichle, 2007). In addition, the identified networks resemble regions identified by task-fMRI, structural connectivity studies and lesion studies (e.g., see Honey et al., 2009; Tavor et al., 2016).

In task fMRI, task engagement is related to high frequency changes in BOLD activation levels; in R-fMRI, low frequency (<0.1Hz) fluctuations in BOLD activity are examined, in the absence of any cognitive task (Cordes et al., 2001). Anatomical connectivity examines the physical interactions between two anatomical areas, estimating the underlying direct causal connection between regions (Gong et al., 2009). On the other hand, Functional connectivity (FC), as assessed using R-fMRI, does not allow the investigation of causal influences, but aims to study the interaction between brain regions by looking at linear temporal correlations. The assumption here is that remote areas that show similar low frequency fluctuations, or in other words correlation in their BOLD timeseries, are functionally connected and belong to the same functional network (Biswal, Yetkin, Haughton, & Hyde, 1995; He & Liu, 2012).

In sum, FC methodology can be used to examine the intrinsic functional organisation within the brain. This implies the study of brain networks, comprising spatially separated but functionally linked anatomical regions that are continuously and spontaneously communicating. In the imaging study described in Chapter VI, resting-state fMRI methodology is used to examine brain networks in healthy and clinical populations.

4.1 R-fMRI value in stroke

Evidence has accumulated indicating the value of connectivity analysis in identifying disturbances in correlation patterns of spontaneous brain activity in clinical populations (Fox & Greicius, 2010). For instance, it has become increasingly apparent that a full understanding of behavioural deficits after stroke will require a complete description not only of lesion topography but also of remote connectivity abnormalities (Siegel, Shulman, & Corbetta, 2017). Changes in functional interactions between cortical areas have been demonstrated to mirror structural changes following stroke (e.g., see review by Grefkes & Fink, 2014). R-fMRI has demonstrated to be sensitive enough to detect a breakdown in frontoparietal connectivity in neglect (He et al., 2007), with the degree of recovery from the pathological bias that correlated to a return toward a normal pattern of functional connectivity (Siegel et al., 2017).

A major pragmatic benefit, and the main justification for using resting state fMRI in this thesis, is that for some modalities there are special demands; e.g., the ability to follow task instructions in task fMRI. Instead, resting state fMRI removes the burden of performing a task in the scanner, as it can be performed virtually in any population with relatively little setup (Biswal et al., 1995). Task-based fMRI studies with less impaired individuals somewhat limits sensitivity to detect disease-related changes, and leads to difficulty in generalisation of the findings of these studies to the wider patient population (Fox & Greicius, 2010). By allowing for a broader sampling of patient populations, R-fMRI has the strong advantage of

rendering the study more inclusive and the cohort studied more representative of the general population.

Also, one area for which R-fMRI is particularity well suited, is for monitoring treatment response. It is indeed possible to examine the effect of a clinical intervention by studying connectivity before and after treatment, or to compare network response to the administration of a treatment or a placebo (Fox & Greicius, 2010).

4.2 Concurrent tDCS-fMRI

Recent methodological advances have laid the ground for further harnessing the potential of connectivity analysis by making it possible to deliver brain stimulation in an MRI environment. By leveraging the complementary strengths of the two techniques, it is possible to shed light on the neural underpinnings of tDCS effects with regard to large-scale networks across the entire brain. Indeed, neuroanatomical tDCS effects may be relatively direct, but might also operate on upstream and downstream components of a functional network involving distant cortical and subcortical sites (Filmer, Mattingley, & Dux, 2019).

To date, only a few studies have administered tDCS intrascanner to examine stimulation-induced changes in brain activity at the stimulation site and in distant brain regions. The vast majority of these studies have been conducted in young adults. These showed widespread connectivity changes during the application of tDCS (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Li, Violante, Leech, Hampshire, et al., 2019; Stagg et al., 2014).

Considering that brains of younger and older adults are different (e.g., see Hedden & Gabrieli, 2004), it is possible that brain stimulation may affect brain networks differently. A common finding of the available studies is that older subjects show reduced functional connectivity of the brain main networks compared to young adults (see review by Sala-Llonch, Bartrés-Faz, & Junqué, 2015).

A small number of such studies were conducted in populations affected by brain pathology, such as traumatic brain injury (Sharp et al., 2011) and patients with frontotemporal dementia (Rytty et al., 2013). At the time of writing, this approach has never been used to explore network response to tDCS in individuals with focal brain injuries.

By using a multimodal approach that combines fMRI and brain stimulation, the research reported in this thesis aims to increase our understanding of the impact of a single dose of brain stimulation on cognitive function (Chapters IV and V) as well as functional connectivity (Chapter VI) in younger, older and stroke populations. This approach is merited in the sense that it may guide interpretation of the well-known inter-individual variability in response to tDCS (Wiethoff et al., 2014). By understanding the different network response induced by tDCS in younger, older and clinical populations, it may be possible to adapt stimulation approaches to these specific groups.

4.3 tDCS-fMRI acquisition

For a systematic review exploring the methodological parameter space of concurrent tDCS-fMRI, see Esmaeilpour and co-workers (Esmaeilpour et al., 2020). For the imaging study described in Chapter VI, the tDCS apparatus (described in Section 3.3) was moved to the scanner preparation room to deliver tDCS in an MRI environment. The two DC stimulators were kept outside the MR shielded room. Direct current was transported into the scanner room via two ethernet cables (one on each side of the penetration panel) after being filtered from RF noise by two filter boxes, one positioned in the operator room and one inside the scanner bore, behind participant's head and connected to the electrodes via the custom MR-compatible wire. The tDCS-fMRI set-up used in this work is shown in Figure 3.8.

During resting-state scans, a monitor located inside the MR room was set to black and participants were instructed to keep their eyes closed and let their mind wander without falling asleep, trying to remain as still as possible.

Resting state functional MRI was obtained at two time points: during real and during sham stimulation (each run lasting for 10 minutes and separated by 10 minutes).

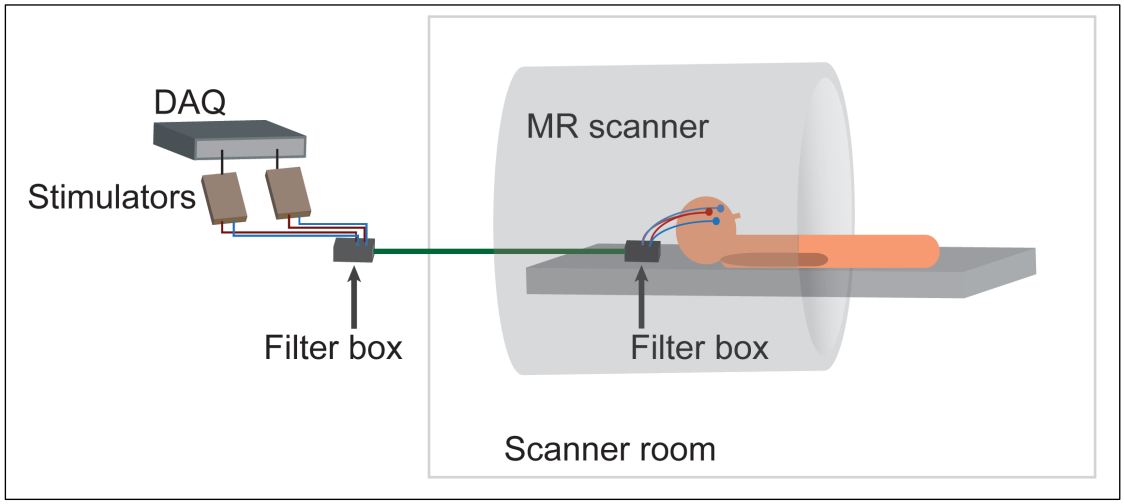


Figure 3.8: tDCS-fMRI setup

4.4 Functional imaging pre-processing

The signal strength over time for a given voxel (i.e., a 3-D pixel) is called the BOLD timeseries or timecourse. Before any statistical analysis on the BOLD timecourse could be carried out, the following pre-processing steps were performed using FSL Version 5.0.10 from the FMRIB (Oxford's Centre for Functional Magnetic Resonance Imaging of the Brain) Software Library (Smith et al., 2004).

4.4.1 Image conversion

All (anatomical and functional) images from healthy and clinical populations were converted from DCOM to NII format using FSL dcm2nii. The converted images were visually inspected for artefacts and large bias fields.

4.4.2 Anatomical images

Image reorientation. The MPRAGE and T2 nii.gz files with best resolution and contrast were reoriented to the standard space using fslreorient2std. Images were inspected to make sure the axes were flipped consistently for all participants.

Brain extraction. Registration of functional images to anatomical images is more robust when non-brain parts of the image (typically a minority in functional images whilst abundant in high-resolution MR images) are removed from anatomical images (Smith, 2002). To this aim, the Brain Extraction Tool (BET) available as part of FSL was used to eliminate non-brain tissue such as scalp, marrow and eyeballs from the structural images T1 and T2 (Smith, 2002). The algorithm used by BET has been tested on thousands of datasets from a wide variety of scanners and of MR sequences. For stroke patients, who may have large lesions within the right hemisphere of their brain, BET parameters were adapted manually until the most optimal brain extraction was achieved. For each participant, the output of BET was inspected by overlapping it with the original T1 (e.g., see Figure 3.9), which allowed the identification of eventual large/consistent errors. The output of BET constituted the input for registering functional to anatomical images.

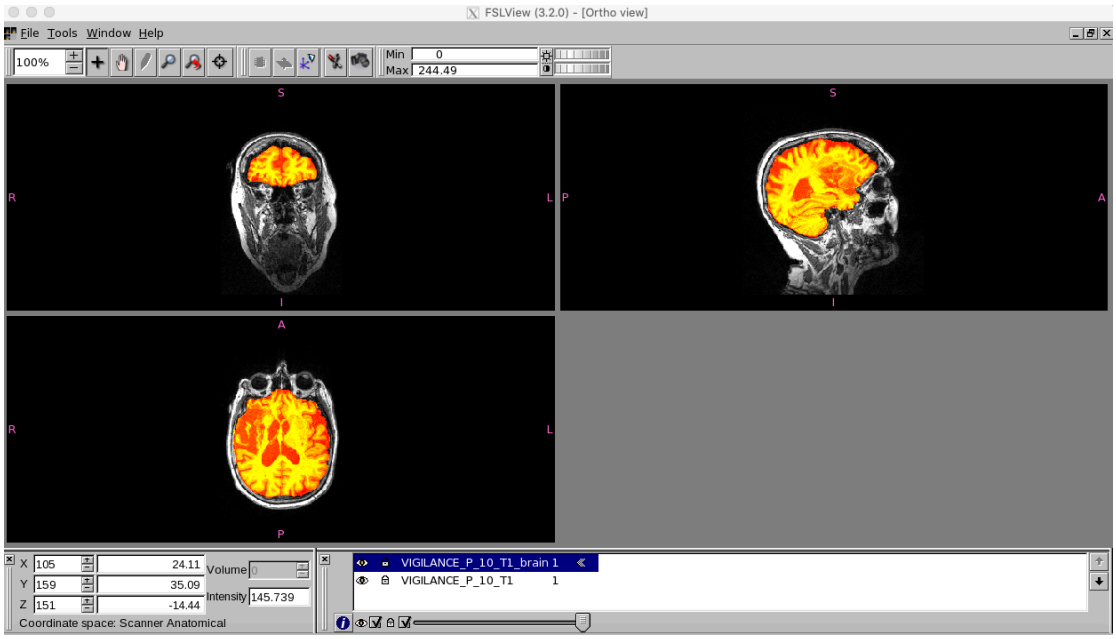


Figure 3.9: Visualisation of the result of the Brain Extraction Tool for Patient 10
The output of BET (red-yellow scale) is overlapped with the T1 (grey scale) using FSLview.

4.4.3 Resting state data quality control

Subject head-motion is undesirable in fMRI studies (Friston, Williams, Howard, Frackowiak, & Turner, 1996), producing changes in the timecourses of resting state MRI data that are not adequately countered for by the use of compensatory strategies such as spatial registration and motion regression (for a thorough characterisation of these effects, see Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). Quality control of resting state functional MRI data was performed using the tool Fsl_motion_outliers, which detects timepoints in the fMRI dataset that have been corrupted by large motion. For each fMRI run (2 runs per participant), motion-contaminated frames were identified using the metric Framewise Displacement (FD), commonly used as an instantaneous head-motion measure (Power et al., 2012). A criterion for excessive motion was set to FD of > 0.5mm in more than 15% of volumes (i.e. if more than 45 out of the 300 total volumes were affected by motion), as per other clinical studies (e.g., Carriere, Lopes, Defebvre, Delmaire, & Dujardin, 2015; De Simoni et al., 2017; Manza, Zhang, Li, & Leung, 2016). This censoring criterion was uniformly applied to all resting state functional MRI data (healthy individuals and patients) before any functional connectivity computations.

4.4.4 Independent component analyses (ICA)

Independent component analysis (ICA) is a model-free voxel-based approach than can be used to explore functional imaging data. It is a data driven approach that decomposes the signal into a set of features called components (Bijsterbosch et al., 2017). ICA was performed using the FSL tool MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) (Beckmann, DeLuca, Devlin, & Smith, 2005).

4.4.4.1 Single-subject ICA

At the individual level, ICA was used to decompose a whole-brain resting state BOLD dataset into spatially independent components (McKeown et al., 1998), which are typically a mixture of signal and noise components. Noise sources are typically head motion and cardiac and breathing cycles.

ICA setup. Each run of fMRI data was conventionally pre-processed as follows: motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002), slice timing correction interleaved, spatial smoothing using a Gaussian kernel filter of 5mm and high-pass temporal filtering of 0.01 Hz, co-registration with BETted T1. Single-subject ICA was then performed, and components identified.

ICA classification. In order to produce a 'clean' denoised version of each individual dataset, all components related to noise need identifying and removing from the data. This was performed using a semi-automated classifier, FMRIB's ICA-based Xnoiseifier (FIX) (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014), which applies an algorithm that can classify signal from noise components in a new dataset following a training phase. The results of the semi-automated classifications were manually inspected and eventually adjusted by examining the available pieces of information: the spatial maps, the time courses and the power spectrum of the time course. (Figures 3.10 and 3.11).

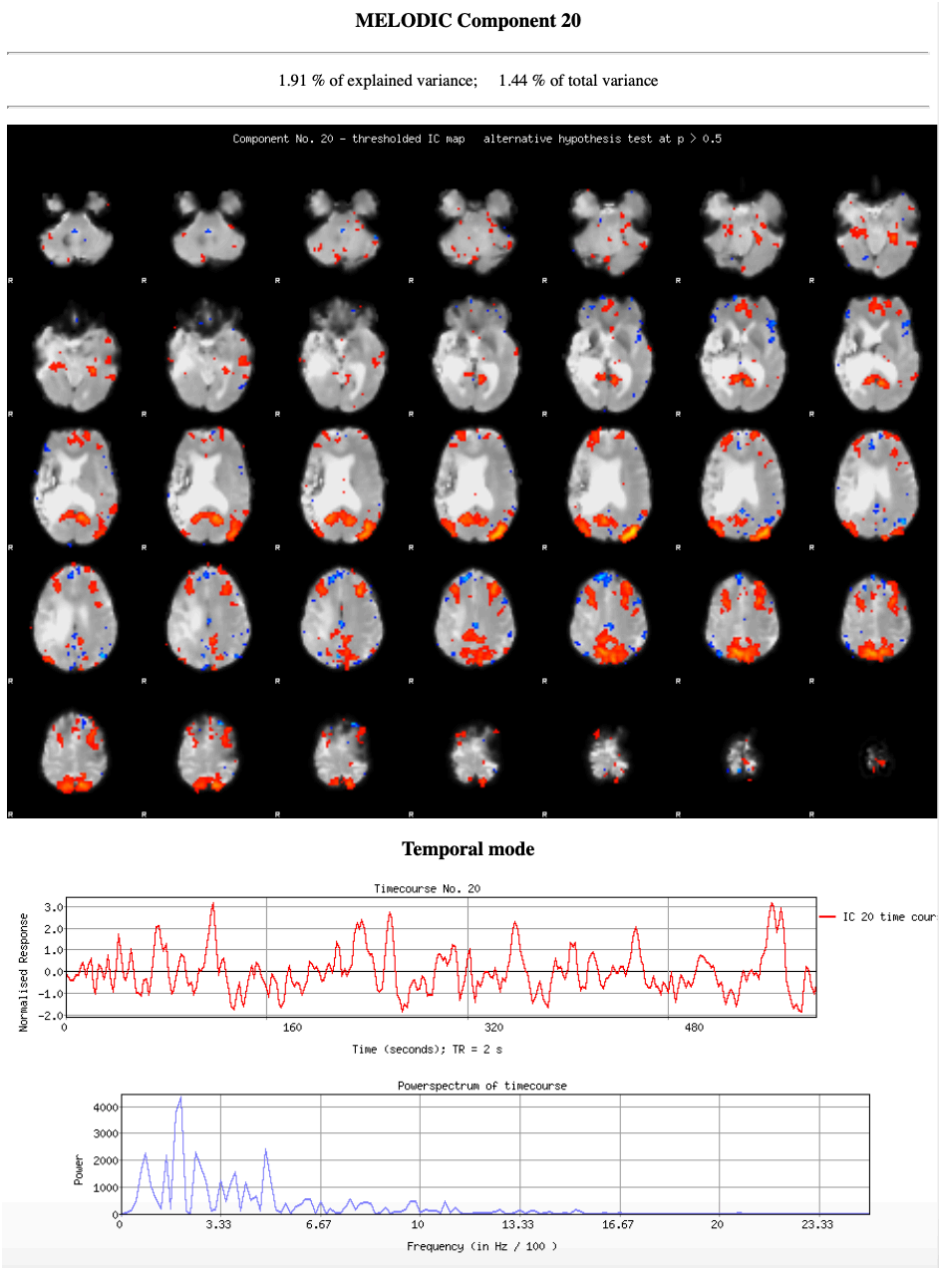


Figure 3.10: Example of a signal component identified by ICA for patient 13
Spatial maps, time courses and power spectra are shown.

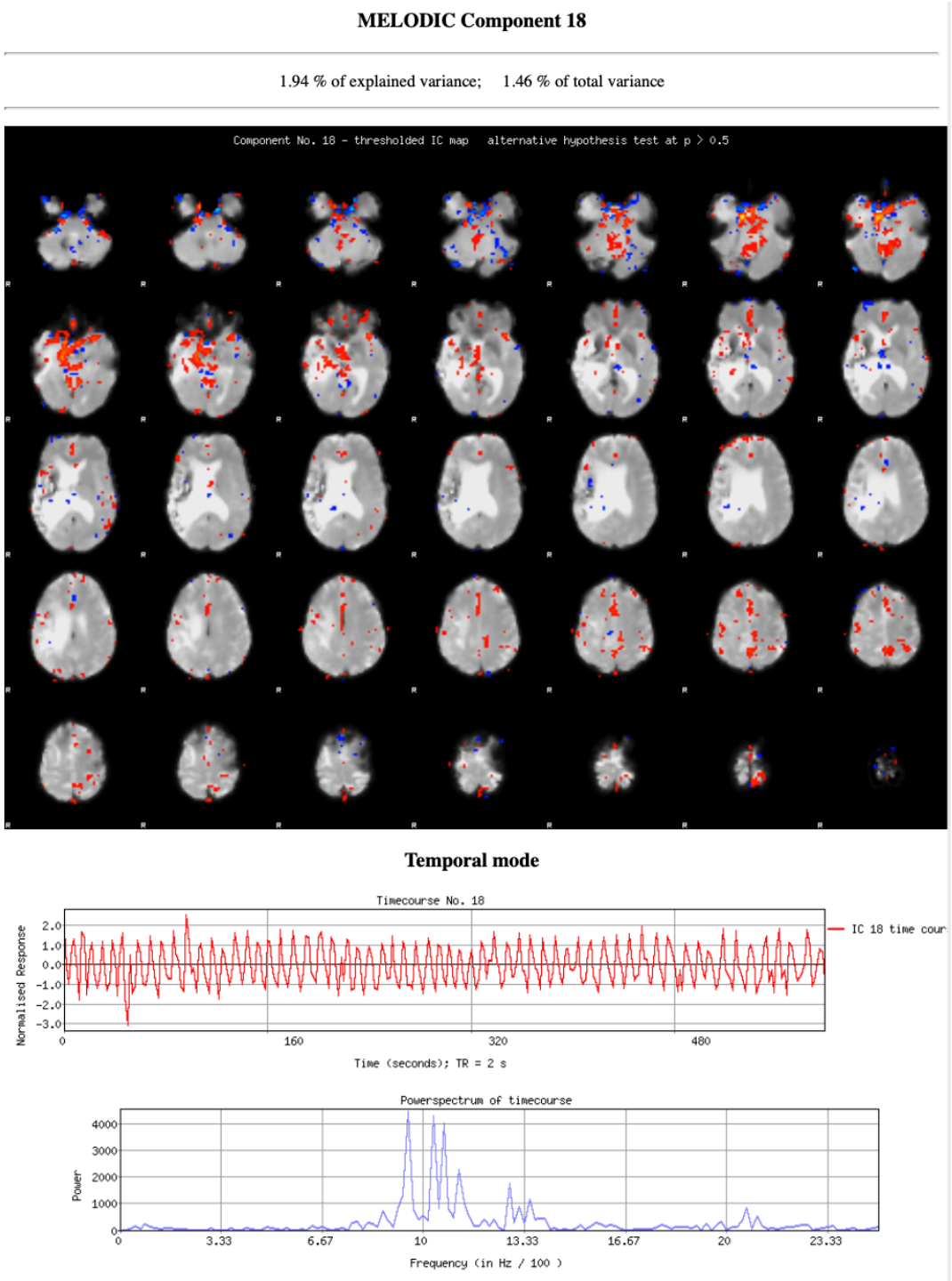


Figure 3.11: Example of a noise component identified by ICA for patient 13
Spatial maps, time courses and power spectra are shown.

4.4.4.2 Specific considerations for FC analysis in stroke

Functional connectivity analysis in stroke patients has particular challenges due to the presence of a lesion (Yourganov, Fridriksson, Stark, & Rorden, 2017). These individuals are likely to have high values of functional connectivity between lesioned areas because physiological noise is sampled from the same pool of necrotic tissue. As these participants are also likely to have behavioural deficits, this leads to paradoxical results when poor behavioural performance is associated with high functional connectivity (Yourganov et al., 2017). In order to isolate the sources of variance in functional data that are lesion-driven, components that showed spatial overlap with the lesion were removed following the method proposed by Yourganov and co-workers, whose study included participants with large unilateral lesions at the chronic stage of post-stroke recovery – similar to the sample of individual tested in the present work (Yourganov et al., 2017). The FSL MELODIC package was used to compute the Z-scored spatial maps of the independent components, which were thresholded at $p < 0.05$ and compared with the lesion mask for that participant. Since both the lesion mask and the thresholded independent component map were binary images, the Jaccard index (the number of voxels in the intersection divided by the number of voxels in the union) was used to quantify the amount of spatial overlap. If the Jaccard index was > 5%, the corresponding component was deemed to be significantly overlapping with the lesion mask. This step removed an additional 1-2% of data – most noise components had already been identified by FIX.

ICA clean-up. All identified noise components (including those originating from the lesion) were removed from each voxel's time series.

5 Conclusions

The brain stimulation setup outlined above will be employed for all the studies described throughout subsequent chapters. The preprocessing pipeline that has been described here was used to preprocess functional imaging data collected as part of the imaging study presented in Chapter VI, Section 4.
This methodology was specifically optimised to allow testing of healthy participants across the life span as well as individuals affected by stroke.

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CHAPTER IV:
Efficacy of targeted tDCS application across the life span

1 Introduction

1.1 Vigilant attention across the life span

The majority of the available empirical evidence indicates that vigilant attention declines as we age (see Chapter I, Section 2.3 and, for a recent review, Fortenbaugh, DeGutis, & Esterman, 2017). For instance, Mani and co-workers showed that, among 19-82 year old adults who completed a continuous performance test, task accuracy decreased as participant age increased (Mani, Bedwell, & Miller, 2005). Fortenbaugh and co-workers arrived at similar findings with a larger pool of participants (n=10,000), which allowed for a more precise modelling of age-related differences, with the ability to maintain accurate performance peaking in the early 40s and starting to decline soon afterwards (Fortenbaugh et al., 2015). However, less evidence is available to elucidate whether an overall deterioration in vigilance is also accompanied by a time-dependent vigilance decrement, which is regarded by some researchers as the hallmark of sustained attention (Parasuraman, Warm, & See, 1998; Rueckert & Grafman, 1996; Rueckert & Grafman, 1998). This is typically defined as a decline in vigilance with increasing time-on-task, represented behaviourally as a decrease in correct detections of targets or increased RT. Despite this, other investigators regard poor performance on monotonous tasks to be a sufficient indicator of suboptimal vigilant attention, without having to demonstrate a vigilance decrement in performance (Manly et al., 2003; Robertson & Garavan, 2004; Wilkins, Shallice, & McCarthy, 1987).

Regardless of whether a decline should be considered the key measure of vigilant attention, the study of performance over time may certainly be helpful to characterise the task: a performance deteriorating with time reduces the possibility of generally poor performance due to task difficulty alone, as participants are able to perform above chance in the first minutes.

1.2 Vigilant attention modulation with tDCS

Researchers have investigated whether transcranial Direct Current Stimulation (tDCS) can modulate the ability to maintain different aspects of attention, with mixed results (for a recent review, see Reteig, Talsma, van Schouwenburg, & Slagter, 2017).

One study showed that anodal tDCS delivered over the left DLPFC interfered with response inhibition on a go/no-go task, but only in allele carriers of a specific gene subtype associated with higher prefrontal dopaminergic activity (Plewnia et al., 2013).
Another study that utilised a tDCS montage involving the anode electrode over the left dorsolateral prefrontal cortex (DLPFC) and cathode electrode over the right supraorbital area, did not reveal any change in task performance (neither RT nor accuracy); instead, a measure of mind wandering increased (Axelrod, Rees, Lavidor, & Bar, 2015). This finding was interpreted by the researchers as potentially suggesting a role for the left executive control network, which includes the DLPFC, in mind-wandering processes.

As discussed in Chapter I, Section 1.3.2, converging evidence showed that the brain networks involved in maintaining attention over time are mainly right-lateralised fronto-parietal networks (Posner & Petersen, 1990). This would support a value in stimulating the right hemisphere to ameliorate vigilant attention. In a recent investigation, the right DLPFC was targeted using the two large pad electrodes of conventional tDCS (Brosnan et al., 2018). This led to an improvement of vigilant attention during active (as compared to sham) stimulation. This study

demonstrated, in two independent groups of healthy older adults, a vigilance-modulating role of prefrontal tDCS on false alarm and accuracy rates on two different vigilance tasks – respectively, one with and one without a strong response inhibition component.
Targeting of the right inferior frontal gyrus was also trialled in a recent investigation (Li et al., 2019). This study featured tDCS delivery whilst healthy adults and individuals with traumatic brain injury were performing the Stop Signal Task, which measures response inhibition. Li and colleagues found that brain stimulation improved response inhibition in control participants, but not in the patient group.

Considering that parietal regions are also part of the right-lateralised brain networks which are key in maintaining an alert state, the targeting of the right parietal cortex has also been attempted by other researchers. In one case, brain stimulation with anode electrode positioned over the right parietal cortex produced slower RT on the final block of a choice reaction time task (which required continuous responses to targets and withholding button-presses to distractors), as compared to anodal electrode positioned over the left parietal cortex (Li et al., 2015). Another study found that oppositional tDCS over the parietal cortices resulted in worsened attentional performance under a high cognitive load condition of a visual tracking task, regardless of the direction of polarity (Roe et al., 2016). A recent preprint suggested that parietal brain stimulation, achieved by positioning anode and cathode electrodes over both right inferior parietal lobules as in oppositional tDCS, did not result in any modulation of performance on a vigilance task with a strong response inhibition component (Coulborn, Bowman, Miall, & Fernández-Espejo, 2020).

Stimulation of the frontal midline has been also selected by other researchers aiming to influence brainstem activity and boost arousal. Miller and co-workers for instance showed no effect of tDCS on accuracy or RT on a go/no-go task (Miller, Berger, & Sauseng, 2015), whereas Mauri and colleagues found that bursts of

stimulation speeded up RT on a continuous performance test (Mauri, Miniussi, Balconi, & Brignani, 2015).

Brain stimulation has also been specifically used in an attempt to counteract the vigilance decrement. Oppositional tDCS applied online at 1 mA for 10 minutes (with anode/cathode electrodes positioned over either prefrontal cortex) has been shown to prevent time-dependent decrement in performance when healthy younger adults were engaged in an air traffic controller simulator (Nelson, McKinley, Golob, Warm, & Parasuraman, 2014). In an offline paradigm, a group of sleep-deprived healthy adults had task accuracy remained stable following 30 minutes of application of 2mA tDCS with anodal electrode over the left DLPFC and cathodal over contralateral deltoid muscle, which was found superior to caffeine in preventing the worsening of performance with increasing time-on-task (McIntire, McKinley, Nelson, & Goodyear, 2017).

In sum, evidence that the application of tDCS may modulate vigilant attention is currently sparse, although there is some evidence supporting a role for tDCS delivered to the prefrontal cortices in vigilance modulation. Interestingly, similar results have been achieved using different current polarities. It is possible that the chosen cognitive task may also play an important role here, as some studies suggested that tDCS may be detrimental to attentional performance when the task involves high attentional demand, potentially overtaxing networks which may then be unable to compensate (Roe et al., 2016). It is worth noting that, with the exception of the work by Brosnan and co-workers, who studied vigilance in older adults (Brosnan et al., 2018), and the study by Li and colleagues in traumatic brain injury (Li et al., 2019), all studies mentioned above have been conducted in samples of healthy younger adults. Therefore, results may not necessarily apply to groups of older adults or individuals affected by brain injury. More research is needed to confirm whether tDCS modulates attention in populations with pathological vigilance impairments.

1.3 Link between vigilant attention and working memory

One cognitive domain that has neuroanatomical links with vigilant attention is working memory (WM); that is, the capacity to flexibly retain and utilise information based on present needs (Baddeley, 2003). The DLPFC seem to exhibit activity in response to working memory-related tasks in a heterogeneous manner, with anticipatory and sustained responses to stimuli (Katsuki & Constantinidis, 2012). Recent electrophysiological evidence supports the role of sustained visuospatial attention in maintaining visual WM contents, potentially by supporting maintenance of representation in an active state (Liang, Chen, Ye, Zhang, & Liu, 2019).

Brain stimulation of the prefrontal cortices has indeed proved able to modulate working memory in a number of studies involving younger participants (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011; Fregni et al., 2005). Reinhart and Nguyen demonstrated a rapid improvement in WM performance following the targeted application of transcranial alternating current (as opposed to tDCS) using a triangular montage similar to the one used in the present study (Reinhart & Nguyen, 2019). Evidence in older adults has also started accumulating. For instance, Park and colleagues showed that bilateral prefrontal stimulation combined with computer-assisted cognitive training improved working memory in a sample of older adults (Park, Seo, Kim, & Ko, 2014).

Thus, there is evidence to suggest that prefrontal tDCS may modulate both working memory and vigilant attention, although it remains unclear if any of such effects are via the same underlying mechanisms. To date, however, researchers have not assessed the effect of prefrontal tDCS on both domains, vigilance and WM, in the same study.

2 Aims

The aim of the present study was to examine whether the ability to maintain attention on a spatial task can be boosted, across the life span, through targeted electrical stimulation of the right dorsolateral prefrontal cortex. This area is part of the right-lateralised brain network involved in maintaining an alert state. Considering the involvement of this region in maintaining information available for processing, the effect of offline tDCS on working memory was also examined as a secondary outcome.

3 Methods

The entire investigation process was conducted according to the principles expressed in the Declaration of Helsinki. All procedures were approved by the Central London Research Ethics Committee and written informed consent was obtained from all subjects.

In a randomised, double-blind, sham-controlled, crossover design study, participants were tested twice, at least 6 days apart, receiving either real or sham stimulation. On each day, participants were told that they may or may not receive brain stimulation. The order of administration of real/sham stimulation was counterbalanced across participants. For two older participants, stimulators were not judged sufficiently charged to deliver real tDCS on the first day of treatment, and sham stimulation was accordingly manually applied, removing researcher blinding. Efforts were made to schedule the two testing sessions at the same time of the day. Participants were asked to avoid consuming drinks containing caffeine for at least two hours prior to the experimental session.

3.1 Participants

The same experimental design was used to test two groups of healthy participants: a younger group and an older group.

3.1.1 A priori justification of sample size

Group sizes for the study were arrived on the basis of the study by Nelson and co-workers, which showed tDCS-related improvement in vigilance levels in a sample of healthy younger volunteers (Nelson et al., 2014). The sample size estimation was carried out (in collaboration with a Consultant Statistician and the NIHR Research Design Service) based on the expectation of finding a similar effect size to that reported by Nelson and co-workers, who reported a highly significant result (p=0.001) of single dose tDCS in 19 healthy younger participants. An estimate of effect size is needed to run a power calculation. As the effect size was not reported in this paper, partial eta squared was derived using G*Power (http://www.gpower.hhu.de/) assuming 90% power at 1% significance level, two-tailed, for a sample size n=19. Partial eta squared is the proportion of variance associated with an effect that is not explained by any other variables included in the model; in other words, it is a measure of how strong an association is. The estimated effect size of 0.98 is a large effect size, indicating a substantive finding, as per previous benchmark values (Cohen, 1988; Field, 2013). This was then used to calculate an a priori sample size, assuming an alpha of 0.05 and power of 0.9. The proposed number for G*power was n=14, and I aimed to recruit at least 18 participants per group to account for possible participant dropout, allowing 25% attrition in each stimulation stream. This power calculation was used to derive sample size for all participant cohorts, i.e., healthy younger and older adults (current Chapter) as well as stroke patients (Chapter V).

3.1.2 Inclusion and exclusion criteria

Participants had no previous history of psychiatric and neurological illnesses (including epilepsy) and denied the presence of any metal implants in the upper part of the body. Full inclusion and exclusion criteria for the study are listed below (modified from the latest document approved by the ethics committee, Protocol_V4, 12th February 2019, pages 7-8).

Inclusion criteria:

- Age >18 years old.

Exclusion criteria:

- Major co-morbid conditions or pregnancy - although there are no data on the negative effects of tDCS on the foetus, pregnant women were excluded as a precaution;
- Severe scalp skin lesions (i.e., skin with ingrown hairs, acne, razor nicks, wounds that have not healed, recent scar tissue, broken skin, etc.);
- Metal implants in the head and neck (e.g., intracerebral vascular clip) or any electrically, magnetically or mechanically activated implant in the body (e.g., cardiac pacemaker);
- Likely to stop or start any psychoactive medications during the course of their participation in the study (i.e., antidepressants, neuroleptics).

3.1.3 Healthy younger adults

The study involved 18 healthy younger volunteers (mean age 29.11±6.37 years old, range 21-40 years old, F=12, all right-handed) (Table 4.1).

ID	Age	Sex	Handedness	Sleep Real	Sleep Sham	Motivation Real	Motivation Sham	Days between sessions
YC1	33	F	R (100)	7.5	7.5	8.6	7.1	14
YC2	39	M	R (100)	6	5	7.5	9.8	64
YC3	23	F	R (100)	7	7	8.5	9	7
YC4	25	F	R (100)	7	8	7	7	8
YC5	29	F	R (100)	7.5	5	6.6	5.4	19
YC6	27	F	R (100)	7	8	9.4	9.4	28
YC7	30	F	R (100)	7	6	9.5	8.5	56
YC8	33	F	R (100)	6.5	7	6.6	6.5	15
YC9	37	M	R (100)	7	7	9.8	9.9	20
YC10	21	M	R (100)	8	7	7.5	4.5	14
YC11	21	M	R (100)	7	7	7.4	5.6	8
YC12	33	F	R (100)	6	5	9.4	9.5	7
YC13	20	M	R (100)	8	7	5.8	5.3	7
YC14	30	F	R (100)	8	4	9.8	10	9
YC15	34	F	R (100)	7	7	8.3	8.5	49
YC16	21	M	R (100)	8	8	8.5	7.5	20
YC17	28	F	R (100)	7	7	9	9	20
YC18	40	F	R (100)	7	7	9.7	9.1	17
mean	29	/	/	7.14	6.64	8.27	7.87	14

Table 4.1. Descriptive statistics for the 18 healthy younger adults who took part in the present study
Columns show age, sex, handedness, hours of sleep the night before and motivation level (on a vertical VAS from 0 to 10) measured on the day they received real and sham stimulation.

3.1.4 Healthy older adults

26 older adults were enrolled to take part in the study. The majority were healthy and fit adults recruited via a local gym, and a subset of participants came from a recruitment database of individuals who had given their permission to be contacted for research; two were relatives of patients attending the Neurology clinic at Charing Cross Hospital.

Data collection could not be completed for 3 older participants, who did not return for the second session - in the first session, two of them received sham stimulation. Before unblinding, two older adults were excluded from any data analysis due to

inability to engage with task demands and because of a technical issue that caused the computer task to stop during the experiment.

Demographics for the remaining 21 older participants (mean age 66.24±9.30 years old, range 51-81 years old, F=8, left-handed=3, ambidextrous=2) are shown in Table 4.2. All these volunteers were fully engaged with the experimenter and the tasks utilised in the experiment.

ID	Age	Sex	Handedness	Sleep Real	Sleep Sham	Motivation Real	Motivation Sham	Days between sessions
OC1	53	F	R (100)	6	7	8	8	71
OC2	64	M	L (-90)	7	6	8.5	8.5	7
OC3	60	M	R (100)	7	6	10	10	16
OC4	67	M	R (100)	7	7	7.9	8.1	6
OC5	71	F	R (100)	6	7	9.5	9.5	6
OC6	64	M	R (100)	6	8	8.5	9	64
OC7	72	F	R (100)	9	9	8	10	50
OC8	51	F	R (100)	5	5.5	9	9.1	29
OC9	75	M	L (-90)	5	6	7.5	8	11
OC10	62	F	R (50)	6.5	5	10	10	7
OC11	68	M	R (100)	7	6	9	9.7	7
OC12	58	M	R (100)	6.5	7.5	8.5	7.7	35
OC13	55	M	R (100)	7	7	8	8.2	7
OC14	69	F	R (100)	7	7	5	9.9	9
OC15	66	F	R (50)	8	8	9.5	9.5	21
OC16	65	M	R (100)	6.5	9	9.4	9.2	20
OC17	55	M	R (100)	12	9	9	9.2	15
OC18	87	M	R (100)	6.5	6	8.8	9.4	14
OC19	71	F	R (100)	7	5.5	6.7	7.3	21
OC20	81	M	R (100)	9	9	7	8.5	4
OC21	77	M	R (100)	6	6	9.2	9.1	8
mean	66	/	/	7.07	6.98	8.43	8.95	20

Table 4.2. Descriptive statistics for the 21 healthy older adults who took part in the present study

Columns show age, sex, handedness, hours of sleep the night before and motivation level (on a vertical VAS from 0 to 10) measured on the day they received real and sham stimulation.

3.2 Experimental timeline

Demographic details were obtained, and consent given, at the beginning of Session 1. Participants were asked how many hours they had slept the night before. In addition, they were instructed to rate their motivation level on a vertical Visual Analogue Scale (VAS) composed of a 100mm length vertical black line, by placing a single horizontal mark across the scale at a point which they felt represented their level of engagement in the experimental procedure. In two different versions of the line, the top and lower extremities represented minimum and maximum level and vice-versa (Appendices III and IV). This measure was included given the evidence that motivation can impact on attention levels (Libera & Chelazzi, 2006). It was used to test for within-subjects differences between subjective motivation levels on the two sessions. Sleep and motivation levels are reported in Tables 4.1 and 4.2. Head measurement were then taken, and electrodes were positioned on the scalp (see Section 3.2.1 for full tDCS set up).

The full outline of each experimental session, which lasted for about 1.5 hours, is summarised in Figure 4.1.

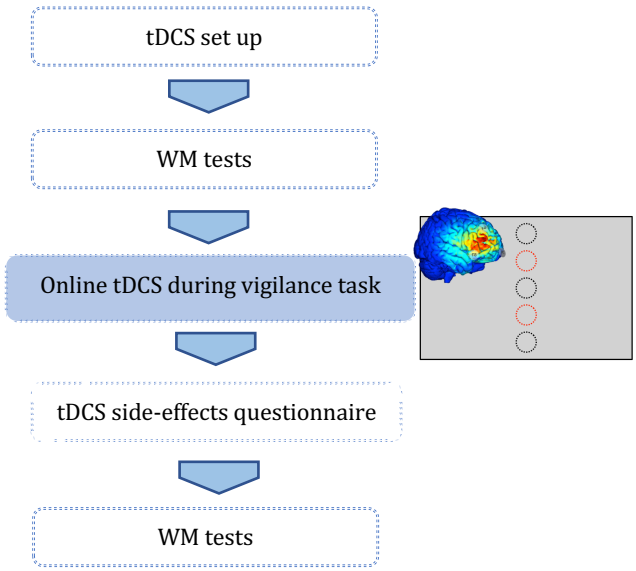


Figure 4.1: Outline of the experimental procedure

Briefly, participants were asked to perform two computer tasks measuring working memory, administered in randomised order prior to tDCS application. Real or sham tDCS was then started concurrently with the vigilant attention task. When these were completed, participants were debriefed about any potential side-effect of stimulation (Appendix V), and invited to repeat the two working memory tasks. All experimental tasks are described in detail in Section 3.3.

3.2.1 tDCS setup

In order to target the right lateralised brain networks involved in maintaining attention over time, a precise, focal stimulation of the right DLPFC was implemented. Full details regarding the tDCS montage, including rationale, apparatus and setup, are provided in Chapter 3, Section 3. Briefly, a triangular montage with anodal electrode over scalp coordinate F4 and two cathode electrodes over F8 and FP2 defined by EEG 10-20 was used to constrain the current in the area between the electrodes. A computational model confirmed that with this montage the peak electric field strength was over the right DLPFC.

Before performing any experimental tasks, electrodes were applied to the participant's head and kept in position using a layer of conductive gel. Impedance was checked and corrected for, being always kept below 10 kΩ. Participants had the opportunity to become accustomed to skin sensations triggered by tDCS application during a habituation phase, where stimulation was delivered for 10s at half intensity followed by 10s at full intensity.

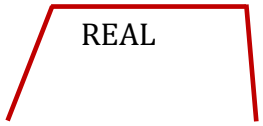
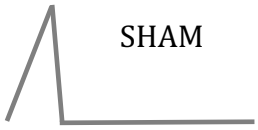
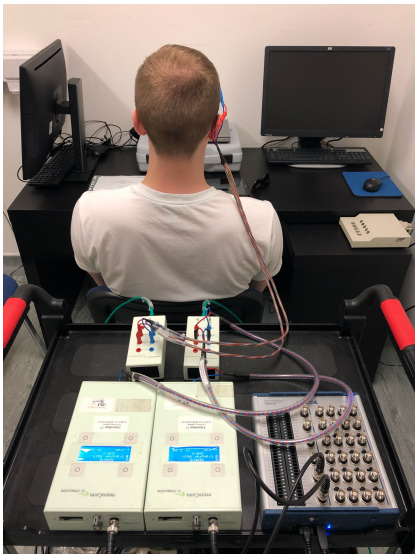
Session (>6 days apart)	Day 1	Day 2
tDCS habituation phase: 10s half intensity, 10s full intensity		
tDCS: real and sham stimulation in counterbalanced order		
Full expt setup: online tDCS, with stimulation administered during task		
tDCS tolerability	Side-effects questionnaire	Side-effects questionnaire

Figure 4.2: Summary of brain stimulation setup for the study
Participants attended the laboratory on two occasions, at least 6 days apart. After a habituation phase, brain stimulation was delivered online, i.e., whilst participants were engaged in a computer task that measured vigilance levels. The order of real and sham stimulations was counterbalanced between sessions: if participants had received real stimulation in the first session, they received sham in the second session and vice versa. Task was performed in dimmed light conditions (not shown).

Targeted stimulation of the right DLPFC was delivered whilst participants performed a computer task that measured the ability to sustain attention to spatial locations (modified from Malhotra, Coulthard, & Husain, 2009; see below for further details). In real tDCS, brain stimulation was delivered with a ramp of 10s up to 1mA, full intensity stimulation for 10 minutes and a ramp down over 0.5s. Sham tDCS consisted of the ramp up and down stages only. tDCS was always administered online, i.e., during the first 10 minutes of the vigilant attention computer task (see experimental setup shown in Figure 4.2), described in the next section.

3.3 Experimental tasks

3.3.1 Vigilance task

Vigilant attention was assessed with a go/no-go computer task controlled using E-prime 2.0 (Psychology Software, Inc.) and running on a 14-inch screen laptop (HP EliteBook 8460p), at a viewing distance set to ~56cm. The decision to use this task was motivated by the broader aim of assessing the potential of targeted tDCS to modulate vigilance after right-hemispheric stroke. Patients with such lesions tend to be severely affected by persisting vigilant attention impairments. The task used in this study constituted an adapted version of a task that has shown already to be able to elicit a vigilant decrement in the relevant population, i.e., right hemispheric stroke patients with neglect (Malhotra et al., 2009). Previous work by Malhotra and colleagues indeed showed that this attentional task, as opposed to a task that does not involve discriminating targets from distractors and that does not include a spatial component, reliably elicits a vigilance decrement in this clinical population (Malhotra et al., 2009).

The new, extended version of the task used here lasted for 15 minutes (approximately twice as long as the original version), without any breaks. It was pilot tested to ensure it was feasible and well tolerated by patients, and it was considered appropriate to the specific purpose of examining vigilant level and vigilance decrement in right-hemispheric stroke. The rationale behind prolonging

the task was to maximise the chances of observing a time-on-task decline in performance, and examining the effect of brain stimulation on such a decline, particularly in the patient group (see Chapter V). Importantly, lengthening the task reduces the risk of a ceiling effect in healthy individuals. Finally, in the present study, brain stimulation was delivered for 10 minutes following on from previous work (e.g., see Nelson et al., 2014); a task outlasting the stimulation period would also allow exploring the after-effects of stimulation on vigilant attention (Figure 4.4).

In the vigilant attention task, participants were presented with a sequence of black letters (consisting of 'A', 'B', 'C', 'D' or 'E', each ~15 × 15 mm), displayed sequentially on a uniform grey background. Letters were presented at one of five possible locations along the vertical meridian of the screen (Figure 4.3). The vigilance task therefore incorporated a spatial component: subjects were asked to respond when targets appear in previously specified locations on the vertical meridian. It required monitoring the location of letters, regardless of their identity. Task engagement involved both spatial and nonspatial (vigilant) attentional processes, and appears to require interplay between fronto-parietal attentional networks (Corbetta & Shulman, 2011). The explicit spatial component incorporated in the task was not spatially lateralised, to avoid the possibility that neglect patients would have difficulty in encoding contralesional stimuli. This is not directly relevant for the study described in this chapter, rather for the patient study described in Chapter V. The vigilance task utilized the first five letters of the alphabet. However, it required participants to ignore the identity of the letters and respond to their location. In the original work by Malhotra and colleagues, of which the task described here represented an extension, the authors did not observe any vigilance decrement when patients were presented with a control task where they had to respond to the identity of the letters, ignoring their location in space (Malhotra et al., 2009).

Participants were asked to respond as quickly as possible, by pressing the central button on the Eprime response box, when they saw a letter at a target location on

the screen (shown as red circle in Figure 4.3), and withhold responses to distractors, i.e. letter presented at non-target locations (shown as black circle in Figure 4.3).

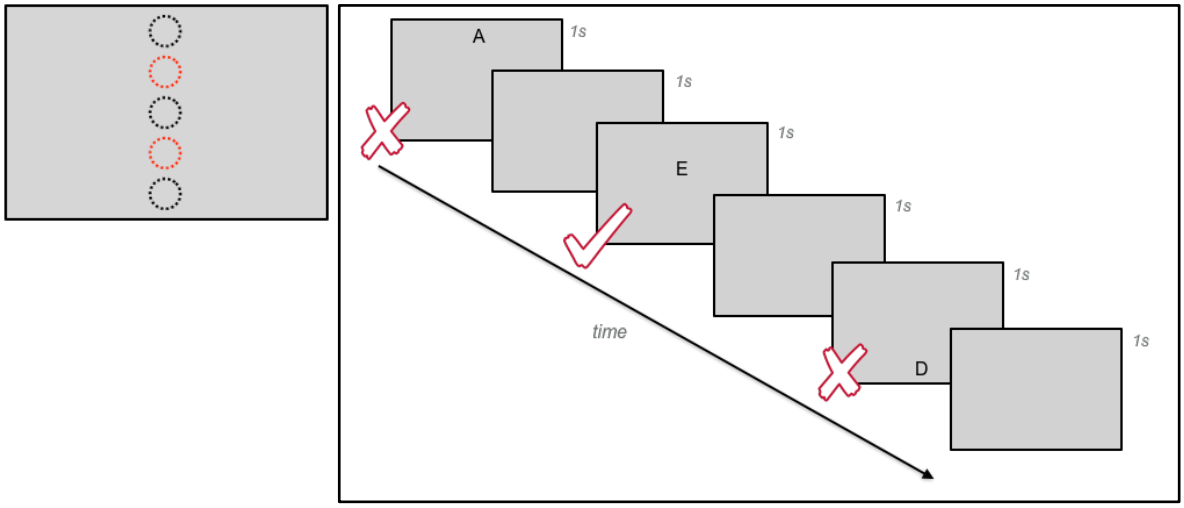


Figure 4.3. Schematic representation of the vigilant attention task
Left panel: The five possible locations along the middle vertical meridian where a letter could appear on the screen. Broken-line circles indicate potential positions: targets locations are shown in red, non-target locations are shown in black. If a letter appears in either of the two locations in red, a button press is required for a correct response. If a response is made when the letter appears in any of the other three locations (in black), that was considered a false alarm.
Right panel: Sequential test display for the task. The first test display shows a letter appearing at one of the non-target locations; the third shows a letter at a target location; the fifth shows a letter at a non-target location.

The paradigm comprised 450 trials in total, with 180 targets and 270 non-target stimuli presented over the time period. Presentation order was randomised as follows: 5 different letters were displayed in one of the 5 possible locations for 90 times, in random order. Letters were displayed every 2s, remaining on the screen for 1s. Correct responses and reaction times (RT) were recorded.
All participants completed a practice run-through of the vigilance task with the researcher, until accuracy was at 100% for 10 consecutive trials. When familiarised, they performed the full task in a quiet, dimmed room to avoid distractions. They received either tDCS or sham stimulation for the first 10 minutes of the 15 minutes task (Figure 4.4).

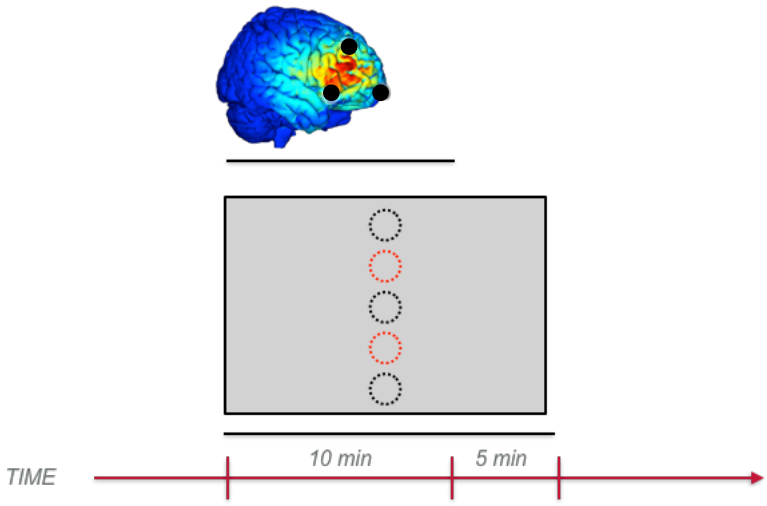


Figure 4.4. Timing of tDCS application during task engagement
Targeted brain stimulation over the right DLPFC was administered during the first 10 minutes of a computer task lasting for 15 minutes.

3.3.2 N-back

To examine working memory effects of tDCS, participants also carried out two tasks before and after receiving brain stimulation. One of them was a numerical n-back task, which is a popular measure of working memory in cognitive neuroscience, requiring on-line monitoring and active manipulation of the memory trace (Kirchner, 1958). In the 2-back version of the task, participants are presented with a stream on numbers and are asked to respond when the digit on the screen matches the digit that appeared two trials before (Figure 4.5).
The version of the task that was used had been designed using the Psychtoolbox (Brainard, 1997) for MATLAB (Mathworks, Release 2012b) as part of another study (Violante et al., 2017). Participants were presented with a single digit (numbers 1-9), one at a time, and were instructed to respond as quickly as possible by pressing the space bar with the index finger of their dominant hand whenever they noticed a repetition of a digit from two positions earlier in the sequence. Each digit (Arial font style, white on black background, height = 2 degrees of visual angle) was presented for 500ms, followed by a fixation cross displayed for 1500ms. Out of 270 trials, 67 (25%) were targets. The experiment lasted for 10 minutes. All participants underwent practice rounds until a threshold of 75% accuracy was reached, before moving to the experimental task.

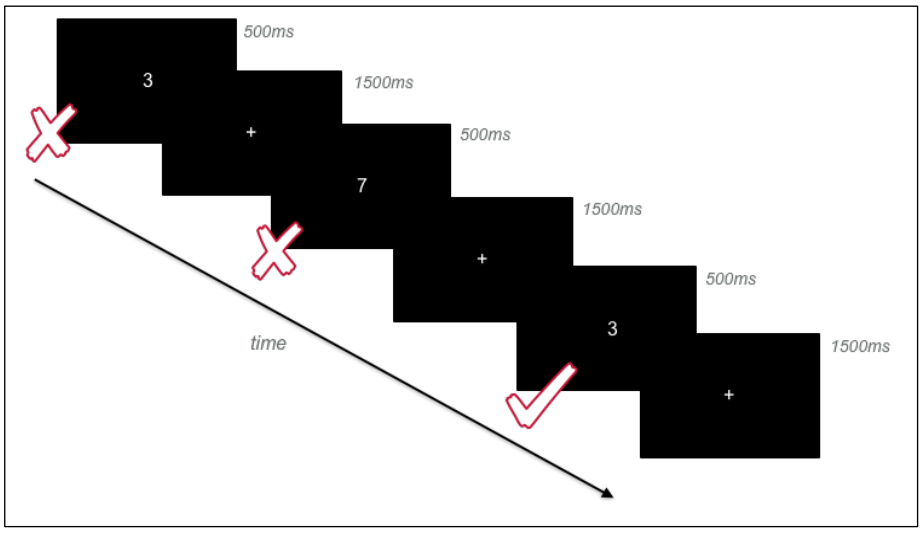


Figure 4.5. Schematic representation of the 2-back task
Participants were presented with a stream of digits and were asked to respond by pressing the space bar every time the number on the screen matched the one that appeared two items before.

3.3.3 Card pairs

The card pairs test is a computerised task that measures the ability to link up two items in memory: the identity of an object and its location. This task, adapted from Owen and co-workers (Owen et al., 2010) by Dr Adam Hampshire at Imperial College, was administered on a Samsung 10' tablet. Participants were shown a set of cards and asked to remember the shape on each. The cards were then flipped over and participants had to identify pairs of cards with identical shapes on them. It scaled up in difficulty according to the participant's performance, with number of cards to remember increasing with correct answers. Performance was indicated by the average number of cards correctly remembered in 3 minutes. Data for this task were not analysed as part of the present thesis.

3.4 Outcome measures

Perception of stimulation was examined by asking participants to state, immediately after having performed the vigilance task, whether they thought they had received any brain stimulation. In addition, they were asked to complete a questionnaire to evaluate possible discomfort and side-effects induced by tDCS (Appendix V) (Brunoni et al., 2011). Specifically, they were asked to rate in

intensity, on a scale from 0 to 4 (with 0 being not at all, and 4 being the strongest sensation they could possibly imagine) the following sensations: tingling, itching, burning, scalp pain, acute change in mood and sleepiness. Results of the analysis performed on perception of stimulation and side effects, for both healthy adults and stroke patients, are reported in Chapter V, Section 5.

My hypothesis was that tDCS would improve performance on the computerised attentional task. The outcome measures analysed for the vigilance task were mean RT, RT variability, errors (omission and commission errors), accuracy, target sensitivity (d') and response bias (C), detailed in the sections below. These are all standard measures that are analysed in vigilant attention tasks.
For the n-back, a subset of these were explored and analysed: mean RT, rates of omission and commission errors and accuracy.

3.4.1 Reaction times

Mean reaction times (RT) were reported in milliseconds (ms). An absolute cut-off of 200ms was used as the lower limit on reaction times in order to eliminate trials containing RT that were likely to represent anticipated responses - these represented the 0.22% of the total number of trials for two participants. Only reaction times for correct responses to targets were analysed.

3.4.2 RT variability

RT variability refers to an inconsistency in the speed of responding which has been argued to reflect a set of abnormally slow responses during a task (Klein, Wendling, Huettner, Ruder, & Peper, 2006; Russell et al., 2006). They are thought to reflect the presence of momentary lapses of attention, during which cognitive resources are diverted towards unrelated processes (Smallwood & Schooler, 2006; Weissman, Roberts, Visscher, & Woldorff, 2006). A coefficient of RT variation was calculated using the formula:

$$CV = RTstandev/RTmean$$

3.4.3 Errors

Both the vigilance task and the n-back task included targets as well as non-targets, permitting the measurement of two types of error: omission errors and commission errors. These were both recorded, and in addition total number of errors was computed. An omission is when participants fail to respond (i.e. do not make a button press) to a target stimulus. A commission error (or false alarm) refers to occurrences when participants (incorrectly) respond to a distractor stimulus. In the case of the vigilance task, a false alarm consisted of responding to letters displayed in non-target locations. In the case of the n-back task, this consisted of a button press when the number on the screen did not match two numbers before.

3.4.4 Accuracy

Accuracy was calculated as a proportion of correct responses, including correct response to targets and correct withholding of response to non-targets.

$$\text{Accuracy} = (\text{correct hits} + \text{correct rejections})/\text{total trials}$$

3.4.5 d prime

D prime (d') is a measure of target sensitivity derived from signal detection theory, which can be applied to any paradigm whereby two stimulus types have to be discriminated (Macmillan & Creelman, 2004). Signal trials involve the presentation of targets; noise trials involve the presentation of distractors. *d'* is estimated by the difference of standardized hit rates and false alarm rates, with hits being the probability of responding 'yes' to signal trials and false alarms the probability of responding 'yes' to noise trial (Stanislaw & Todorov, 1999).

$$d' = z(\text{hits}) - z(\text{FA})$$

d' is therefore a z-transformed score based on the standard normal distribution which assumes equal-variance distributions. Higher d′ means higher sensitivity, whereas d′ closer to zero means chance-level performance.

This statistic takes into consideration hits and false alarms, whereas accuracy incorporates hits and correct rejections. In cases whereby presentation rates in the experiment are different, and an unequal number of targets and non-targets are presented, accuracy and d' may not directly correspond to each other. For instance, in the vigilance task there are 40% signal trials and 60% noise trials, and a participant always responding 'noise' (i.e., not responding at all) would have 60% accuracy but a *d'* of 0, meaning that the subject was not able to discriminate signals from noise, thus performing at chance levels.

3.4.6 Response bias (C)

The presence of any response bias, that is the general tendency to respond 'yes' or 'no', was analysed using the response criterion (C) (Stanislaw & Todorov, 1999). C is defined as the distance between the criterion and the neutral point, where neither response (yes/no) is favoured. Deviations from the neutral point are measured in standard deviation units. C was found using the formula (Macmillan, 1993):

$$C = z(\text{hits}) + z(\text{FA})/2$$

If participants use a liberal criterion, they show a bias towards 'yes' responses (negative values); if they use a conservative criterion, the bias is towards 'no' responses (positive values).

3.5 Data analysis

Statistical analyses were performed using SPSS Statistics v26.0 (IBM, 2019). For each experimental task, the shape of data distribution within each group (younger and older adults) and for each stimulation condition (real and sham) was assessed using the Kolmogorov-Smirnov test, as reported in Appendix VI. In cases whereby the assumption of normality was violated (i.e., accuracy and all types of errors), scores were log transformed to address skewed data. Non-transformed values are however reported and plotted in the results sections, for easier interpretation of findings. After each log transformation, a Kolmogorov-Smirnov test was repeated and was not significant in all cases, ensuring normality. The assumption of

homogeneity of variance was tested using Levene's test, which ensured equal variances between sessions in all parameters.

4 Results

The Wilcoxon signed-rank test was used to compare hours of sleep and motivation levels between sessions. The amount of sleep declared before the real or sham tDCS stimulation evaluations was not significantly different at the two administration times ($p>.05$); motivation levels were also not significantly different between sessions ($p>.05$) (Table 4.3).

	active tDCS	sham tDCS	Related samples statistic
Sleep	7.10±1.19	6.82±1.22	z=-1.413, p=.158
Motivation	8.36±1.21	8.45±1.45	z=-.453, p=.650

Table 4.3. Mean±SD amount of sleep (hours) and motivation (VAS scale)
Sleep and motivation levels were assessed at the beginning of each experimental session for all participants (n=39).

4.1 Vigilance: overall performance

An index of overall performance on the vigilance task was calculating by averaging across all 450 trials. Data were analysed using a series of repeated measures analyses of variance (ANOVA), with Group (younger vs older) and Order (real-sham vs sham-real) as between-subjects factors, and Stimulation (real vs sham) as within-subjects factor. RT, RT variability, total number of errors, accuracy, target sensitivity and response bias were included as dependent variables in the model. For significant effects (p value $\leq .05$), effect sizes were calculated using partial eta squared (η_p^2) and observed power was computed using alpha=.05. Bonferroni post-hoc corrections were used to explore significant effects and interactions.

4.1.1 Reaction times

The ANOVA of mean RT revealed a main effect of Group [$F_{(1,35)}=5.973$, $p=.020$, $\eta_p^2=.146$, observed power=.662]: older adults were significantly slower on this

task as compared to younger adults (respectively, mean 509.036, SEM 10.527; mean 455.942, SEM 11.418), regardless of brain stimulation (Figure. 4.6). No other main effects or interactions emerged [all p values >.05].

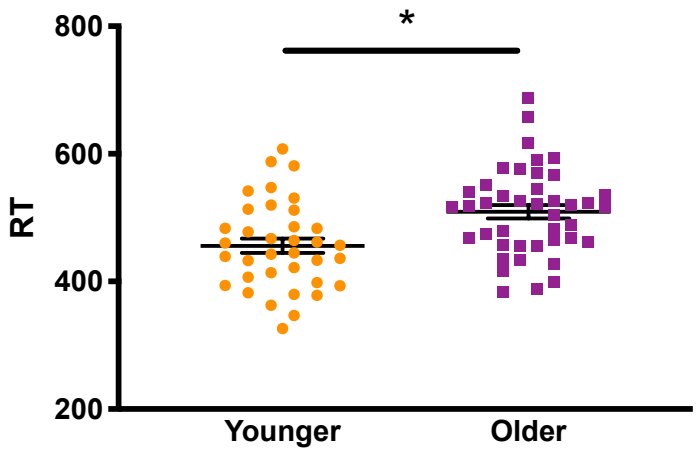
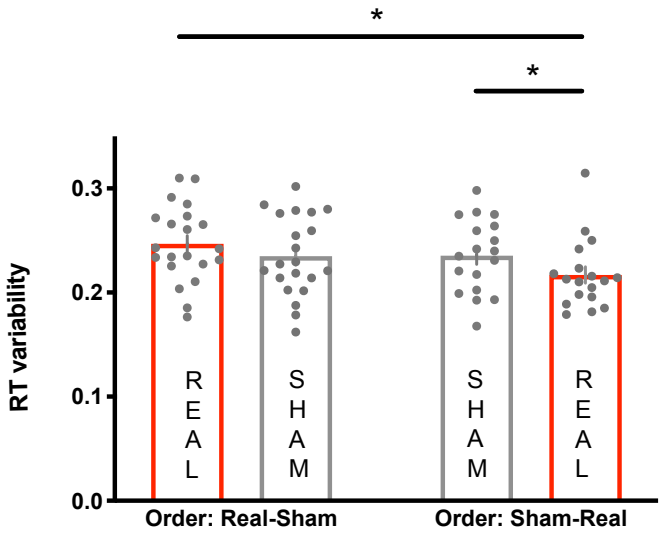


Figure 4.6. Mean RT for younger (tangerine dots) and older (plum squares) adults
On average, older adults were significantly slower on the task, as compared to younger adults. Mean RT with SEM are represented by black bars.

4.1.2 RT variability

The ANOVA of the coefficients of RT variability revealed a significant interaction between Stimulation x Order [$F(_{1,35})$=6.763, p=.014, η_p^2=.162, observed power=.715] (Figure 4.7). In the group of participants who received sham in the first session and real stimulation in the second session, RT were significantly less variable during real (mean 0.217, SEM 0.008) as compared to sham tDCS (mean 0.236, SEM 0.009) [p=.037]; in cases where real preceded sham, a comparable RT variability was registered in real (mean 0.247, SEM 0.008) and sham stimulation sessions (mean 0.235, SEM 0.008) [p=.146]. RT during real tDCS were also found to be significantly more variable when real was administered as a first treatment (mean .246, SEM 0.008), whereas in the group that received real after sham RT variability was smaller (mean .217, SEM 0.008) [p=.016]. RT variability during sham tDCS was not significantly different between groups [p=.917].

All other main effects and interactions were not significant [all p values >.05].



4.7. RT variability during real and sham stimulation for participants receiving treatment in different orders: real-sham and sham-real
Lower RT variability was registered during real as compared to sham stimulation, but only for the group of participants who received tDCS in the second session. Here, stimulation may have potentially produced a facilitation in performance that interacted with task familiarity. When brain stimulation was administered in the first session, the advantage in RT consistency levels as compared to sham was not observed, as in the second sessions RT variability remained stable.

In sum, older adults were slower on this task. TDCS appeared to produce a facilitation in the consistency of individual's speed of responding which interacted with treatment order. Lower RT variability was found during real as compared to sham stimulation, but only when tDCS was administered in the second session. This finding suggests that RT remained more stable when the effect of tDCS was combined with that of task familiarity.

4.1.3 Total errors

Data for the total number of errors were log transformed for analysis. The ANOVA showed a significant interaction between Stimulation x Order [$F(_{1,35})$=16.246, p=.000, η_p^2=.317, observed power=.975] (Figure 4.8). Participants who received sham in the first session and real in the second session, made significantly fewer errors during real (mean 4.611, SEM 1.029) as compared to sham stimulation (mean 8.611, SEM 1.613) [p=.001]. In cases where real preceded sham, a

comparable amount of errors was made during real (mean 5.762, SEM 1.060) and sham stimulation (mean 4.286, SEM 0.903) [p=.076]. In terms of the errors made during sham stimulation, the two groups differed: those who received sham first made more significantly more errors (mean 8.611, SEM 1.613) than those who received sham in the second session (mean 4.286, SEM 0.903) [p=.014]. No difference between groups emerged for the real stimulation condition [p=.474].

Main effects and other interactions did not reach statistical significance [all p values >.05].

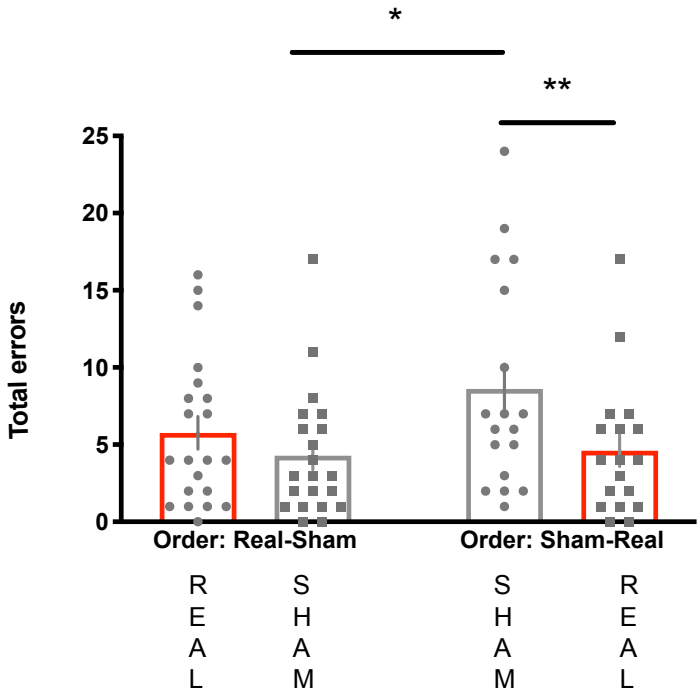


Figure 4.8: Total number of errors during real and sham stimulation for participants receiving treatment in different orders: real-sham and sham-real
Participants in the sham-real group made significantly more errors during sham, as compared to real stimulation. In the other group, no significant changes across sessions emerged. The two sham sessions were also different from each other, and significantly more errors were done when sham was administered first.

Briefly, when tDCS was delivered on day 1, the number of errors remained stable in a succeeding session; when brain stimulation was delivered on day 2, when they were more familiar with the paradigm, participants made fewer errors on the task, as compared to a previous session without stimulation.

4.1.4 Omissions

Log-transformed omissions constituted the input for the repeated measures ANOVA. A significant main effect of Stimulation emerged [$F_{(1,35)}$=4.344, p=.045, η_p^2=.110, observed power .527], with fewer omissions made during real (mean 0.385, SEM 0.150) as compared to during sham stimulation (mean 0.795, SEM 0.230) (Figure 4.9). Other main effects and interactions did not reach statistical significance [all p values >.05].

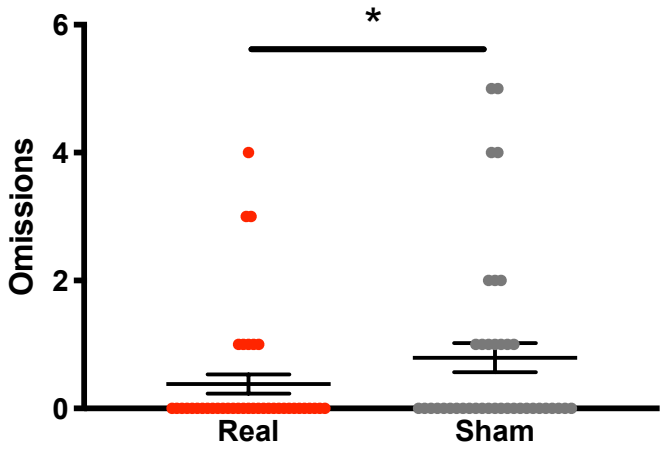


Figure 4.9. Total number of omissions made during real and sham stimulation by healthy participants
Participants omitted fewer targets when they performed the task whilst receiving real as compared to sham stimulation. Mean and SEM for the number of omissions are represented by black bars.

4.1.5 Commission Errors

Commission errors, or false alarms, comprised the vast majority of errors in healthy individuals. Log-transformed commission errors constituted the input for a repeated measures ANOVA, which showed a significant interaction between Stimulation x Order [$F_{(1,35)}$=11.634, p=.002, η_p^2=.249, observed power=.912] (Figure 4.10). Post-hoc analysis showed that participants who received sham in the first session and real in the second session, made significantly fewer commission errors during real (mean 4.389, SEM 0.994) as compared to sham stimulation (mean 7.611, SEM 1.553) [p=.006]; in cases where real preceded sham,

the number of commission errors made during real (mean 5.238, SEM 0.961) was comparable to those made during sham stimulation (mean 3.667, SEM 0.691) [p=.070]. Similarly to what discussed for total errors, the number of commission errors made by the two groups during sham was significantly different [p=.026], with those who received sham first (mean 7.611, SEM 1.553) making more false alarms than those who received sham afterwards (mean 3.667, SEM 0.691). No difference in the number of false alarms emerged when comparing the two real stimulation sessions [p=.516].

All main effects and other interactions were not significant [all p values >.05].

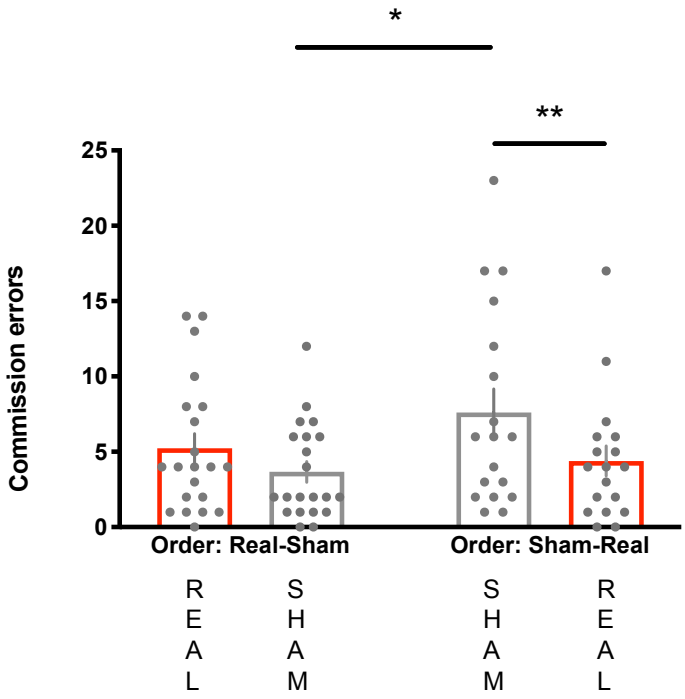


Figure 4.10. Total number of commission errors (i.e., false alarms) made during real and sham stimulation by healthy participants
Participants in the sham-real group made fewer false alarms when performing the task during real, as compared to sham stimulation. The other group of participants (i.e., those receiving real-sham) made a comparable amount of errors during both sessions.

Analysis of false alarms mirrored the findings already discussed for the total number of errors. This is not surprising, considering commission errors constituted the majority of errors.

4.1.6 Accuracy

All participants performed the vigilance task at an accuracy level above 90%. Reverse scored log transformed data for proportion of correct responses were analysed via the three-factorial (Group x Order x Stimulation) repeated measures ANOVA, which revealed a main effect of Stimulation [$F(_{1,35})$=6.651, p=.014, η_p^2=.160, observed power=.708], with greater accuracy levels detected during real stimulation (mean 98.838, SEM 0.164) as compared to sham stimulation (mean, 98.604, SEM 0.210) (Figure 4.11). Such difference in proportion of accurate responses between real and sham tDCS is of small magnitude, as indicated by the effect size which explains 16% of the total variance.

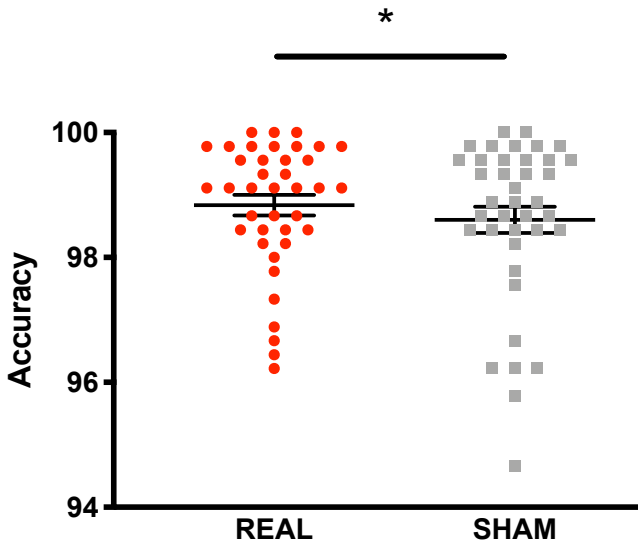


Figure 4.11: Accuracy during real and sham stimulation
Task accuracy was above 90% in all cases. Healthy participants were significantly more accurate on the task when they received real (red dots), as compared to sham (grey squares) brain stimulation. Mean RT with SEM are represented by black bars.

The interaction between Stimulation x Group [$F(_{1,35})$=4.102, p=.050, η_p^2=.105, observed power=.504] was significant, with significant effect of stimulation in the sample of older adults (real: mean 98.794, SEM 0.234; sham: mean 98.476, SEM 0.278) [p=.002] but not in younger adults (real: mean 98.889, SEM 0.235 ; sham: 98.753, SEM 0.323) [p=.707] (Figure 4.12). Other pairwise comparisons for the two conditions did not reach statistical significance [all p values >.05].

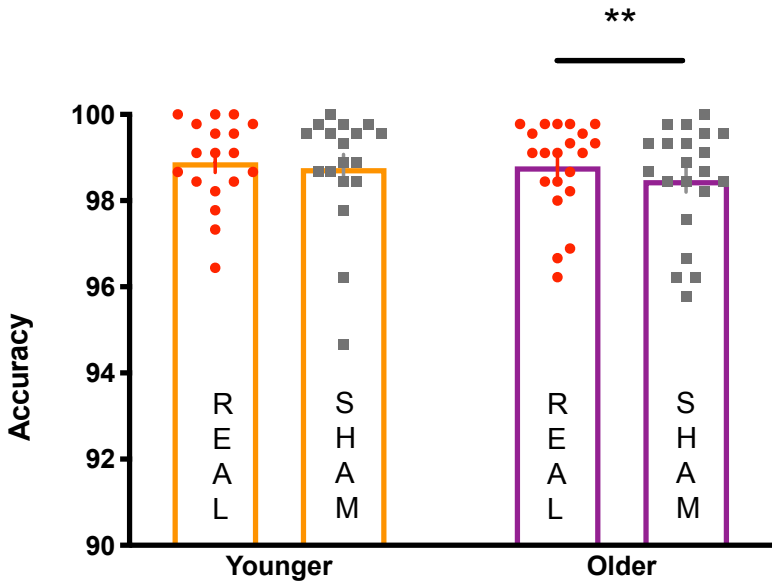


Figure 4.12. Accuracy during real and sham stimulation for healthy younger (tangerine columns) and older (plum columns) participants
The effect of tDCS on task accuracy was more pronounced in the older group.

The interaction between Stimulation x Order [$F(_{1,35})$=16.220, p=.000, η_p^2=.317, observed power=.975] was significant, suggesting a differential effect of stimulation depending on the treatment administration order (Figure 4.13). In cases whereby sham preceded real stimulation, performance during real (mean 98.975, SEM 0.229) was significantly better than during sham stimulation (mean 98.086, SEM 0.358) [p=.000]. Participants who received real followed by sham stimulation, on the other hand, performed the task at similar accuracy levels during both conditions (real: mean 98.720, SEM 0.236; sham: mean 99.048, SEM 0.200) [p=.296]. Also, the two shams were found to be significantly different between order groups [p=.016], with participants who received sham first performing at lower accuracy levels (mean 98.086, SEM 0.358) as compared to those who received sham in the second session (mean 99.048, SEM 0.200). Accuracy levels for the two groups were comparable during real stimulation, regardless of treatment order [p=.478].

No main effects or other interactions reached statistical significance [all p values >.05].

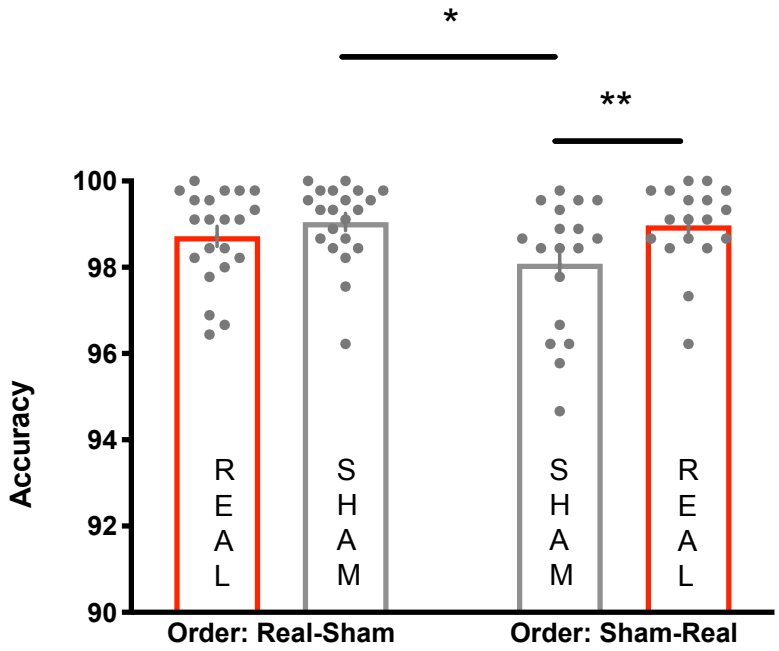


Figure 4.13. Accuracy during real and sham stimulation for participants receiving treatment in different orders: real-sham and sham-real
Participants were significantly less accurate during sham, as compared to real stimulation, when sham came first. Also, they were significantly less accurate than the other group of participants during sham. This suggests that brain stimulation may have had a facilitating effect.

To sum up, these findings indicate that stimulation improved accuracy levels on this task, in line with the results obtained from omission analysis. Furthermore, older adults, who performed at similar accuracy levels as younger participants, appeared to benefit more from the application of brain stimulation. The facilitating effect of stimulation interacted with treatment order. When stimulation came first, it appeared to boost performance to a level which was maintained in the second session. When sham stimulation came first, on the other hand, participants were less accurate; performance nevertheless improved when real tDCS was administered successively.

4.1.7 Target sensitivity (*d'*)

The ANOVA examining target sensitivity (*d'*) revealed a significant interaction between Stimulation x Order [$F(_{1,35})$=14.361, p=.001, η_p^2=.291, observed power=.958] (Figure 4.14). Similarly to what previously discussed for commission

errors and accuracy, participants who received sham in the first session and real stimulation in the second session, had significantly higher target sensitivity during real (mean 4.996, SEM 0.103) as compared to sham stimulation (mean 4.613, SEM 0.121) [p=.000]. In cases where real preceded sham, comparable levels of sensitivity were registered during real (mean 4.862, SEM 0.112) and sham stimulation (mean 4.998, SEM 0.113) [p=.160]. Also, sham sessions were significantly different between groups depending on the treatment order [p=.026], with participants receiving sham first performing at lower sensitivity levels (mean 4.613, SEM .121) than those who received it afterwards (mean 4.998, SEM .113). No difference between the two real sessions emerged [p=.419].

All main effects and other interactions were not significant.

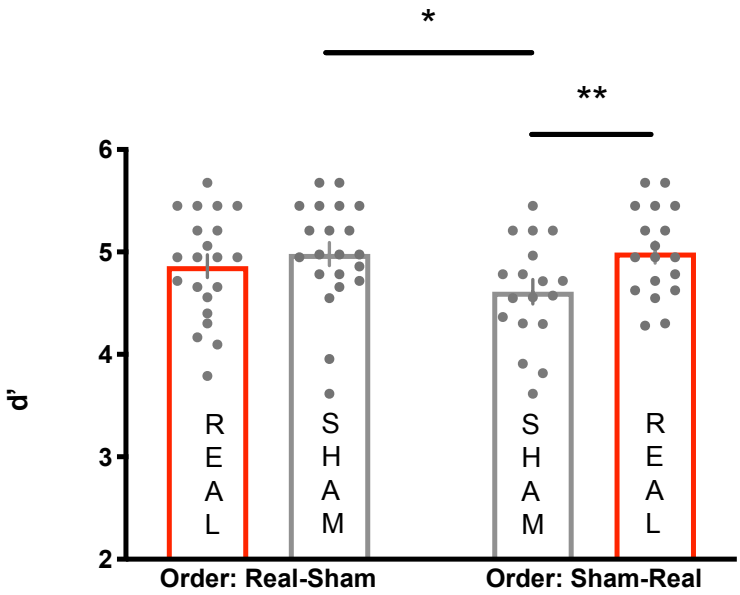


Figure 4.14. Target sensitivity (*d'*) during real and sham stimulation for participants receiving treatment in different orders: real-sham and sham-real
Target sensitivity was significantly lower during sham as compared to real stimulation, for participants receiving treatment in sham-real order. For participants who received real stimulation as the first treatment condition, target sensitivity was at the same level during both sessions. Interestingly, the two shams sessions were significantly different from each other.

To summarise, as regards to the ability to discriminate targets from distractors, brain stimulation significantly affected performance, depending on when it was administered. In parallel with what discussed for other outcome measures, the two groups performed differently during sham stimulation, depending on whether this preceded or followed a session of tDCS. When tDCS was the first treatment, participants performed the task at high levels of target discriminability which was then sustained in a second session.

4.1.8 Response bias

The ANOVA examining for the presence of any response bias (C) revealed no significant main effects or interactions [all p values >.05].
On average, the criterion was located -0.50 standard deviations away from the neutral point both during real and sham stimulation for all participants, with no significant differences between healthy younger and older adults. This ruled out a possible effect of brain stimulation or age on the strategy used by responders.

4.2 Vigilance: decremental performance

To examine how performance changed over time, the task (comprised of 450 trials and lasting for 15 minutes) was divided into three time-epochs, each lasting for 5 minutes. To examine the vigilance decrement, all the outcome measures discussed for overall performance were compared across epochs.

A four-way repeated measures analyses of variance (ANOVA), with Group (Younger vs Older), Order (Real-Sham vs Sham-Real) as between-subjects factors and Stimulation (real vs sham) and Epoch (t1 vs t2 vs t3) as within-subjects factors was the statistical analysis of choice to reveal any effect of time-on-task. As for overall performance, effect sizes for significant effects (p value $\leq$.05) were calculated using partial eta squared (η_p^2) and observed power was computed using alpha=.05. Bonferroni post-hoc corrections for multiple comparisons were used to explore significant effects and interactions.

4.2.1 Reaction times

The four-factorial ANOVA (Group x Order x Stimulation x Epoch) examining RT confirmed the main effect of Group [$F(_{1,35})$=5.991, p=.020]: older adults were slower on the task as compared to younger adults, as discussed above in Section 4.1.1.

A main effect of Epoch [$F(_{2,34})$=11.588, p=.000, η_p^2=.249, observed power=.992] also emerged: participants were slower in epoch 3 (mean 495.923, SEM 0.294) as compared to epoch 2 (mean 483.617, SEM 8.350) [p=.006] and epoch 1 (mean 473.749, SEM 8.019) [p=.002] (Figure 4.15). This suggested that there was an increase in RT towards the end of the task, which was however not modulated by brain stimulation [Stimulation x Epoch: F(1,35)=1.075, p=.347].

All other main effects and interactions were not significant [all p values >.05].

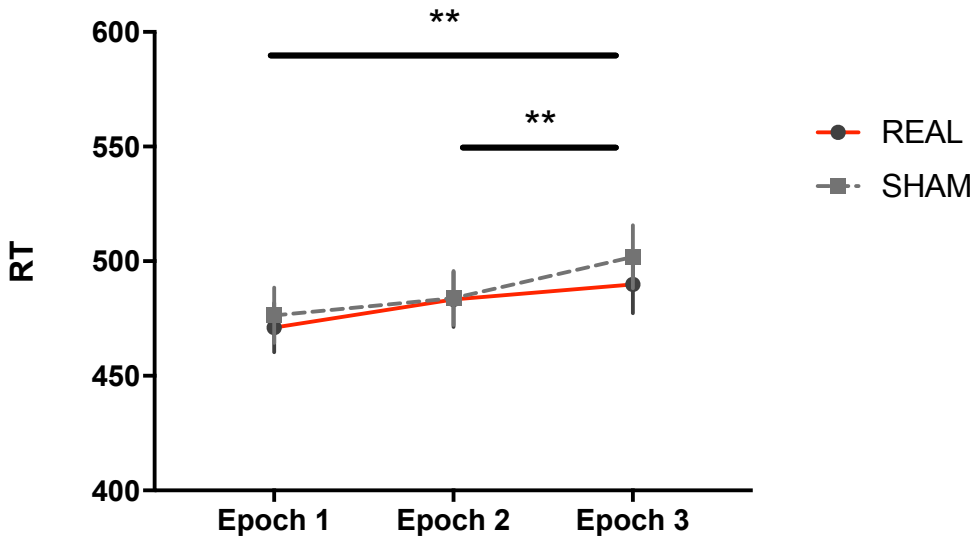


Figure 4.15: Slowing down in RT for healthy adults across epochs
Healthy adults exhibited a vigilance decrement in RT, with higher RT with increasing time-on-task. The slowing down in RT did not appear to be modulated by brain stimulation.

4.2.2 RT variability

The four-factorial ANOVA (Group x Order x Stimulation x Epoch) examining reaction times variability showed a main effect of Epoch [$F_{(2,34)}$=3.494, p=.036, η_p^2=.091, observed power=.635] (Figure 4.16). RT were more variable in epoch 3 (mean .2367, SEM .005) as compared to epoch 2 (mean .2223, SEM .004) [p=.004], but not as compared to epoch 1 (mean .229, SEM .005) [p=1.000]. No statistical difference in RT variability was found between epoch 1 and 2 [p=.431].

The significant interaction between Stimulation x Order [$F_{(1,35)}$=5.008, p=.032] discussed for overall performance (Section 6.1.2) was confirmed.
All other main effects and interactions were not significant [all p values >.05].

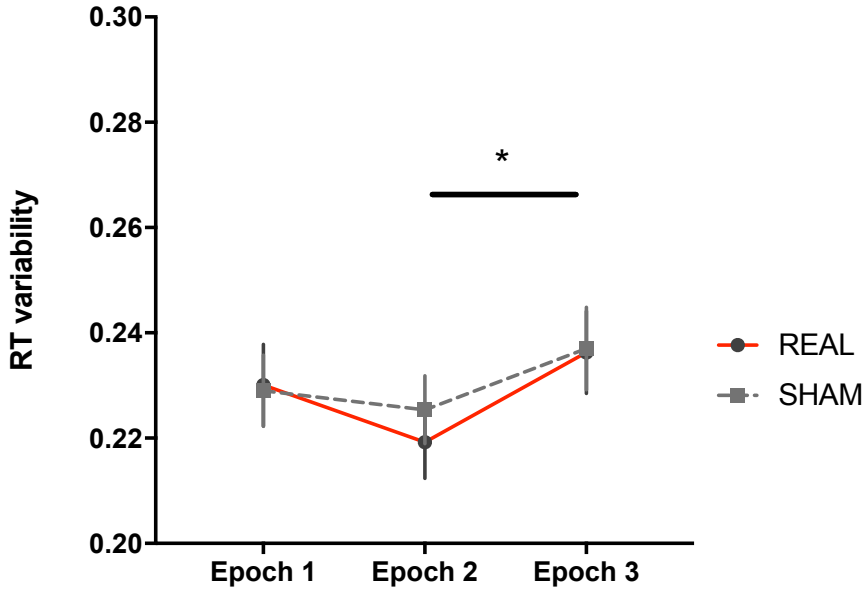


Figure 4.16: RT variability in healthy adults across epochs
RT were more variable in epoch 3 as compared to epoch 2 in healthy participants. No modulation by brain stimulation emerged.

To summarise results on RT, healthy adults were significantly slower during the last five minutes of the task. An increase in the number of apparent lapses of attention, as measured by RT variability, also emerged towards the end of the task. Brain stimulation did not have any effect on these parameters indexing vigilance decrement.

4.2.3 Total errors

A four-factorial ANOVA (Group x Order x Stimulation x Epoch) examining the total number of errors showed a significant Stimulation x Order interaction [$F(_{1,35})$=10.170, p=.003], as discussed above in Section 6.1.3. No other significant main effects or interactions emerged [all p values >.05].

4.2.4 Omissions

The small number of omissions made by healthy participants did not allow for any meaningful characterisation by epoch.

4.2.5 Commission errors

A four-factorial ANOVA examining the total number of errors showed a significant Stimulation x Order interaction [$F(_{1,35})$=15.935, p=.000], as discussed above in Section 4.1.5. No other significant main effects or interactions emerged [all p values >.05].

4.2.6 Accuracy

The four-way ANOVA that was carried out to examine inverted and log transformed accuracy scores showed a main effect of Stimulation [$F(_{1,35})$=7.001, p=.012] and significant interactions between Stimulation x Group [$F(_{1,35})$=4.137, p=.050] and Stimulation x Order [$F(_{1,35})$=15.018, p=.000] – these have been already discussed above in Section 4.1.6.

There were no other main effects nor interactions that were statistically significant [all p values >.05].

4.2.7 Target sensitivity (*d'*)

Using the four-factorial ANOVA, other than the Stimulation x Order interaction [$F(_{1,35})$=13.516, p=.001] discussed above in Section 6.1.4, there were no significant main effects or interactions [all p values >.05].

4.3 Working memory

For the n-back task, data for 18 younger and 19 older adults were available. Data for two older adults (2 males, 67 and 81 years old) were not analysed because they did not pass the practice phase – to keep the structure of the session as similar as possible, they were asked to perform the 1-back version of the task instead, but this was not analysed. Participants performed the working memory task twice, i.e., before and after the administration of tDCS. All scores were analysed via a series of repeated measures ANOVA with Group (younger vs older adults) and Order (real-sham vs sham-real) as between subjects factor and Stimulation (real vs sham) and Session (pre vs post) as a within subjects factors.

4.3.1 Reaction times

The ANOVA of RT showed that all main effects and interactions did not reach statistical significance (all p values > .05).

4.3.2 Omissions

The ANOVA of the log-converted rates of omissions showed a main effect of Session [$F(_{1,33})$=12.689, p=.001, η_p^2=.278, observed power=.933], with participants missing fewer targets in the post- (mean 12.687, SEM 1.851) as compared to the pre-stimulation session (mean 13.350, SEM 1.857).

A significant interaction between Session x Group [$F(_{1,33})$=6.496, p=.016, η_p^2=.164, observed power=.696] emerged: older adults made significantly fewer omissions post- (mean 13.000, SEM 1.601) as compared to pre-stimulation (mean 16.614, SEM 1.672) [p=.000]. All other comparisons for older adults, and all comparisons for younger adults were not significant [all p values >.05].

The interaction between Stimulation x Order [$F(_{1,33})$=8.904, p=.005, η_p^2=.212, observed power=.825] was statistically significant (Figure 4.17). Participants undergoing stimulation in the order real-sham missed fewer targets during the second sham session (mean 11.492, SEM 1.397) as compared to the first real

session (mean 16.119, SEM 1.726) [p=.028]. All other comparisons were not significant [p>.05].

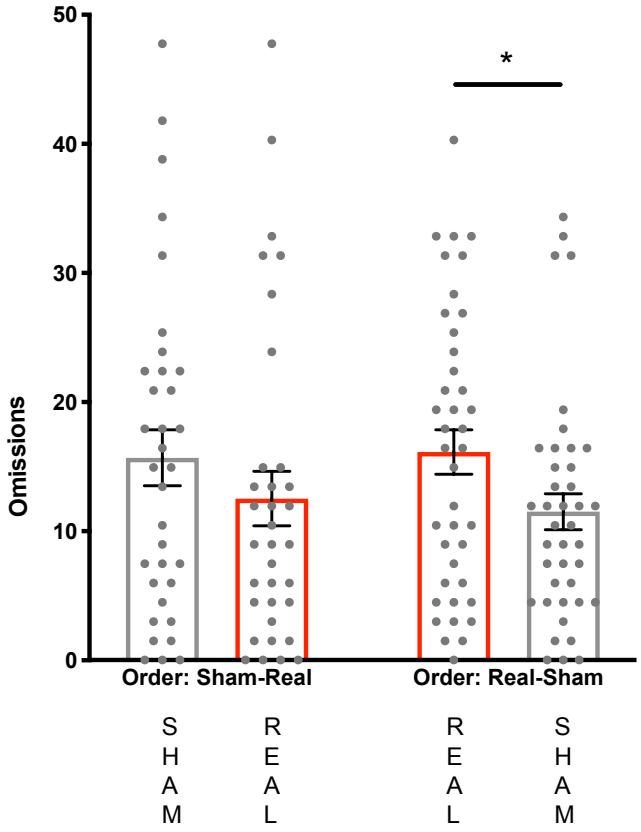


Figure 4.17: Omissions made on the n-back task by the two order groups
Fewer omissions were made during sham stimulation, as compared to real tDCS, by the group who received real tDCS in the first session and sham tDCS in the second session (second two columns), versus the group who received sham in the first and real tDCS in the second (first two columns).

The three-way interaction between Group x Session x Order [$F(_{1,33})$=5.901, p=.021, η_p^2=.152, observed power=.655] emerged as significant. Older adults in the sham-real group missed significantly fewer targets post- (mean 9.422, SEM 1.871) as compared to pre-stimulation (mean 15.951, SEM 2.566) [p=.001]. All other comparisons in older adults as well as all comparisons in younger adults did not reach statistical significance [all p>.05].

No other main effects or interactions came out significant (all p values >.05).

In sum, healthy adults missed fewer targets at post-stimulation evaluations. This effect was more pronounced in the sample of older adults. It was especially older adults in the sham-real group who made fewer errors in the post-stimulation session. Also, fewer omissions were made post-sham stimulation, when this was the second session.

4.3.3 Commission errors

The ANOVA of the log-converted percentages of commission errors showed a significant main effect of Group [$F(_{1,33})$=4.670, p=.038, η_p^2=.124, observed power=.555], with older adults (mean 6.404, SEM 0.639) making significantly more false alarms than younger participants (mean 3.797, SEM 0.406).

A main effect of Session [$F(_{1,\ 33})$=11.599, p=.002, η_p^2=.260, observed power=.911] also emerged, with all participants committing significantly fewer errors post- (mean 4.540, SEM 0.731) as compared to pre-stimulation (mean 5.731, SEM 0.849) on each testing day.

The interaction between Stimulation x Order [$F(_{1,33})$=6.434, p=.016, η_p^2=.163, observed power=.692] was significant (Figure 4.18). Fewer false alarms were made with real stimulation (mean 5.071, SEM 1.001), as compared to sham (mean 6.404, SEM 1.000), by those participants who received sham followed by real tDCS [p=.05]. On the other hand, for those participants who received the treatment in the order real-sham, a comparable number of commission errors were made in the real (mean 5.012, SEM 0.594) and sham session (mean 4.237, SEM 0.577) [p=.135]. Performance during real stimulation and sham stimulation was comparable between treatment orders [p=.788 and p=.109 respectively].

Other main effects and interactions did not reach statistical significance (all p values >.05).

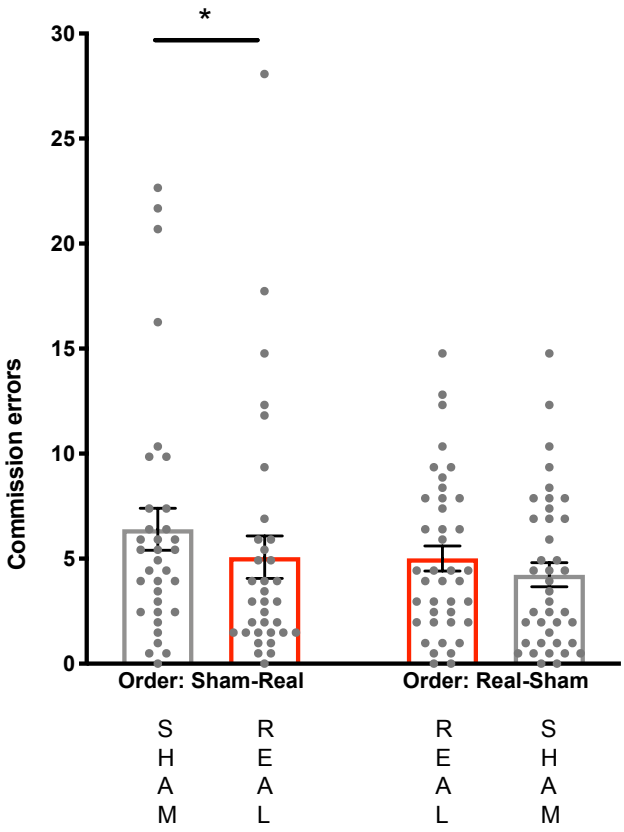


Figure 4.18: Commission errors made on the n-back task by the two order groups
Fewer false alarms were made during real stimulation, as compared to sham tDCS, by the group who received sham in the first and real in the second session (first two columns) versus the group who received real in the first and sham in the second (second two columns).

To summarise results on false alarms, older participants made more commission errors than younger adults. On each day, participants performed better the second time they performed the task. Brain stimulation significantly affected performance, in interaction with treatment order. Participants who received real in the first session, maintained a comparable performance in a succeeding session. Participants who received sham first on the other hand, had their performance improved in the second real session.

4.3.4 Accuracy

The ANOVA of task accuracy revealed a significant main effect of Session [$F(_{1,33})$=4.497, p=.042, η_p^2=.120, observed power=.539], with participants

performing better in the post- (mean 87.152, SEM 1.851) as compared to pre-stimulation session (mean 84.974, SEM 1.857).

A significant Stimulation x Session interaction emerged [$F(_{1,33})$=4.730, p=.037, η_p^2=.125, observed power=.560]. On the day of real stimulation, performance in the pre- (mean 84.268, SEM 1.877) and post -stimulation session (mean 86.809, SEM 1.952) diverged [p=.001], whereas for sham stimulation, pre- (mean 85.680, SEM 1.837) and post-stimulation accuracy scores (mean 87.495 SEM 1.749) were not significantly different from each other [p=.641]. Also, before any stimulation was administered, participants were significantly less accurate on the day they received real (mean 84.268, SEM 1.877), as compared to the day they received sham (mean 85.680, SEM 1.837) [p=.035]. This difference was not present in the second session [p=.383], with comparable accuracy levels achieved after real (mean 86.809, SEM 1.952) and sham stimulation (mean 87.495 SEM 1.749).

This effect appeared to be driven by the older group of participants. The interaction between Group x Stimulation x Session [$F(_{1,33})$=4.005, p=.054, η_p^2=.108, observed power=.493] was at significance level, and explored via planned contrasts. In the sample of older adults, performance significantly improved between pre- (mean 81.147, SEM 2.611) and post-stimulation evaluations (mean 85.860, SEM 2.589) on the day they received real stimulation [p =.000], whereas it was not significantly different between pre- (mean 85.625, SEM 2.030) and post-stimulation (88.138, SEM 1.952) when sham stimulation was administered [p=.441]. Again, performance pre-stimulation was significantly less accurate on the day they received real stimulation (mean 81.147, SEM 2.611), as compared to sham (mean 85.624, SEM 2.030) [p=.006]. In the post evaluation, however, there was no difference in accuracy between the groups [p=.281]. For the younger group, there was no difference between sessions [all p values >.05].

A significant Group x Session x Order [F(1,33)=10.667, p=.003, η_p^2=.244, observed power=.887] was observed. Older adults who received sham-real performed significantly better in the post- (mean 90.578, SEM 1.871) as compared to the pre-

stimulation evaluation (mean 84.048, SEM 2.566) [p=.003]; those who received real-sham performed similarly before (mean 82.904, SEM 2.249) and after (mean 84.396, SEM 2.302) real and sham stimulation [p=.401]. Other comparisons for the older group and all comparisons for younger adults did not reach statistical significance [all p values >.05].

A significant interaction between Stimulation x Order emerged [$F_{(1,33)}$=12.921, p=.001, η_p^2=.281, observed power=.937]: participants who received real stimulation in the first session and sham in the second session, had significantly higher accuracy levels during the second sham session (mean 88.507, SEM 1.400) as compared to the first real session (mean 83.881, SEM 1.730) [p=.003]; in cases where sham preceded real, participants showed comparable levels of accuracy during the sham (mean 84.328, SEM 2.166) and real sessions (mean 87.490, SEM 2.111) [p=.066]. During real and sham sessions, performance was not significantly different between groups [p=.197 and p=.354 respectively].

The three-way interaction between Group x Stimulation x Order [$F_{(1,33)}$=.458, p=.019, η_p^2=.156, observed power=.669] was significant. Older adults who received real stimulation as the first modality, performed better during the sham session (mean 88.127, SEM 1.733) as compared to the real session (mean 79.172, SEM 2.345) [p=.000]; those who received sham in the first session, performed better during real (mean 89.459, SEM 2.338) as compared to the sham stimulation session (mean 85.168, SEM 2.330) [p=.035]. No other comparison was statistically significant in older adults, and in the group of younger adults no significant difference between sessions emerged [all p values >.05]. Overall, this interaction suggests that older adults performed the task better on day two, regardless of stimulation.

Other main effects and interactions did not reach statistical significance [all p values >.05].

To sum up n-back accuracy results, all participants were more accurate the second time they performed the task on each day. Such increase in accuracy levels appeared to be more pronounced following real stimulation. However, considering that on the day they received real tDCS participants started from a significantly lower baseline, they may have had more room for improvement. It appeared that older adults in the sham-real group showed bigger improvements in the post- as compared to the pre-session. Another finding was that older adults had higher accuracy in the second session, irrespective of stimulation.

5 Discussion

The aim of the present study was to assess, using a robust crossover design methodology, the efficacy of the targeted application of prefrontal brain stimulation to improve vigilance in a cohort of healthy adults, a younger group and an older group. The vigilance network was targeted by using a precise triangular montage centred on the right DLPFC. This setup was intended to achieve optimal focality over what is considered a key region for vigilant attention. Brain stimulation was applied during the first 10 minutes of a cognitive task that required participants to maintain concentration over 15 minutes, without any breaks. The effect of offline tDCS on working memory was also examined.

5.1 Vigilant attention across the life span

Results from the vigilance task analysis showed that older adults were generally slower on this task, as compared to younger adults. Performance was nonetheless comparable across the life span in terms of errors, accuracy and target sensitivity on the task. In accordance with existing theories, we expected older participants to perform less well, as compared to younger adults. However, this was not demonstrated using this task, and task accuracy was above 94% for all participants. This may be due to the choice of the present paradigm, motivated by an interest to compare vigilant attention in healthy and clinical populations, but possibly not sensitive enough to detect changes across the life span, reducing the potential to detect an age difference in healthy adults. A previous large-scale study

arrived at similar results, with age differences in RT but not in detection rates using a much longer task, the classic Mackworth Clock Test lasting for 62 minutes (Giambra & Quilter, 1988). McAvinue and colleagues detected an age-related decline in performance using the SART (which requires continuous button presses to frequent targets), with lifespan trajectories characterised by a plateau in young and middle adulthood and a deterioration only later on into older adulthood (McAvinue et al., 2012). To explore this issue further, future studies could consider stratifying sampling, or recruiting participants in older ages only. For the purpose of this thesis, a group of older adults was chosen with the aim of matching the age of stroke patients.

When examining changes in performance over time, a slowing down of responses and an increased variability in RT over time was recorded for all participants, with no difference between younger and older adults. The task used here proved able to elicit a vigilance decrement in healthy populations, but only in reaction times. In terms of accuracy, no worsening of performance over time emerged over the course of the 15 minutes task. These findings are in partial agreement with the previous study by Malhotra and co-workers, who did not observe any change in performance (neither RT nor *d'*) with increasing time-on-task in a sample of older adults performing a shorter version of the task utilised here (Malhotra et al., 2009). It is possible that the longer version of the task used in the present work may have elicited a slowing down in response speed, but potentially a longer version is needed to elicit a decrement of accuracy in healthy populations.

5.2 Effect of tDCS on vigilant attention

Brain stimulation was administered online, i.e., whilst participants were engaged in a computer task that measured attention levels. Response speed on the task was not modulated by the electrical targeting of the right dorsolateral prefrontal cortex. Other measures of task performance, however, proved to be susceptible to tDCS modulation, with participants performing better during real as compared to sham stimulation.

Target detection improved under the effect of tDCS. Interestingly, the effect of brain stimulation on accuracy was significantly more pronounced in the older group. This finding is in keeping with previous literature in the field (see review by Perceval, Flöel, & Meinzer, 2016), and could depend on age-related changes in excitation and inhibition rates and brain morphology (e.g., brain shrinking and increase in cranial cerebrospinal fluid with age), which may influence the intensity and distribution of electric fields (Mahdavi, Towhidkhah, & Initiative, 2018; Thomas, Datta, & Woods, 2018). Future studies that systematically incorporate information about age-associated brain reorganization, could be helpful in claryfying the mechanisms that may render tDCS more effective in older groups.

Another possible explanation for the effect is that older adults were further away from ceiling on the task and may have had more room for improvement. However, this seems unlikely considering that no main effect of Group emerged, suggesting that younger and older participants performed the task at similar accuracy levels.

Analysis of error type showed that fewer omissions were made during real as compared to sham stimulation. In other words, fewer targets were missed when brain stimulation was delivered concurrently with the task.

For total number of errors, number of false alarms, accuracy and target sensitivity, analysis systematically revealed that brain stimulation had an effect that consistently interacted with treatment order. Specifically, when the placebo sham stimulation was administered on the first day of treatment, participants made more errors (including false alarms alone) and showed lower accuracy and target sensitivity, as compared to a second session involving brain stimulation. This is potentially accounted for by a beneficial effect of tDCS. Alternatively, one could argue that participants would perform better on the second day of treatment because of a practice effect. However, even if some task familiarity cannot be ruled out and may have indeed taken place, it is unlikely to have caused the observed facilitation, considering that this pattern was not observed for the other group of participants who received treatment in the reverse order. If practice was the only

critical influence upon performance, an improvement in performance across sessions should have emerged for both groups, regardless of treatment order. However, this is not what the analyses showed. In fact, for the group of participants who received stimulation as the first treatment option, the number of errors made (including false alarms alone), accuracy and target sensitivity all remained stable across sessions.

Different possible explanations could account for this finding. For instance, the effect of tDCS on excitability could be carried over consecutive sessions, so that when participants repeated the task, they are still under the beneficial influence of tDCS. However, the majority of the available experimental evidence to-date shows increased cortex excitability lasting for up to 90 minutes post-stimulation (Nitsche & Paulus, 2001), and a washout period of at least 6 days (median 14 days) was left in-between sessions for the current study. It could be possible that the targeted montage utilised in the present study caused a longer lasting effect of stimulation. The existing literature on high definition tDCS, although limited, supports the notion that the pattern of effects of HD-tDCS fits those of conventional tDCS (Kuo et al., 2013). However, it has been suggested that, after stimulation of the motor cortex, the time course of the respective excitability alterations may differ, for instance with a delayed peak and a longer lasting effects (at least 30 minutes longer) following HD tDCS but not conventional tDCS (Kuo et al., 2013). Evidence for a prolonged effect of one application of tDCS that is extended beyond a few hours is nevertheless scarce. An exception is the study by O'Shea and co-workers that found that the coupling of conventional tDCS with prism exposure was able to induce persisting effect of one application over a follow-up period (18–46 days) (O'Shea et al., 2017).

Considering that a proposed mechanism of action of tDCS involves the induction of LTP and LTD-like phenomena, which also underly memory and learning, it is possible that tDCS may have enhanced learning. A body of evidence showed that anodal stimulation of areas that are specifically engaged in task learning may speed up learning, e.g., Broca's area during a grammar task (De Vries et al., 2010),

the right PPC in a visuo-spatial task (Bolognini, Fregni, Casati, Olgiati, & Vallar, 2010) and the DLPFC during a WM task (Andrews et al., 2011). TDCS and training may interact, although other studies showed that they may not necessarily lead to additive effects (e.g., see Muller-Dahlhaus & Ziemann, 2015). An alternative explanation to that of a carryover of the effect of tDCS could be that, when stimulation is delivered in the first session, it may boost performance to a level that would otherwise be achieved only in a successive session, when familiarity comes into play. In this group, the level of performance exhibited in the first session could be attributable to the presence of brain stimulation. Performance in the second (sham) session, on the other hand, could be influenced by some familiarity with the experimental procedure. It is worth noting that, after a training phase, participants were able to carry out the vigilance task at high accuracy levels throughout the paradigm in both sessions. The finding that performance is stable and does not improve over time reduces the possibility that significant learning is taking place as participants perform the task.

These effects could also be attributed to a baseline difference between groups. It should be noted that a baseline session was not included in the study design, and therefore it is not possible to state with absolute certainly that baseline levels of performance do not contribute to the findings (Learmonth, Thut, Benwell, & Harvey, 2015). However, this seems unlikely because the main effect of group did not emerge as significant in any of the analyses; performance for session 1 is not significantly different between groups.

Irrespectively of treatment order, performance for the two real sessions was comparable between groups. In other words, the effect of brain stimulation combined with task familiarity was not bigger that brain stimulation alone. It was specifically performance for the sham sessions that differed between groups: when sham came first, it could have not been influenced by practice or brain stimulation; when sham followed a session with real tDCS, it may have been affected either by brain stimulation, by some familiarity with the experimental procedure, or by the combination of these – but the contributions of each cannot be dissected in the

current study. One potential way to explore whether there is any practice effect at all with this task would be to have a group of participants perform two experimental sessions both during sham stimulation. Any potential difference between sessions would be due to practice. This group could then be compared to the group who received sham-real, to confirm whether an additional effect of tDCS on top of practice exists.

The effect of stimulation did not appear to diminish immediately after stimulation was stopped. All measures of task performance were comparable across time epochs, with the exception of RT and RT variability which were unaffected by online tDCS.

Reaction time variability, potentially reflecting occurrences of lapses of attention, was at its lowest when brain stimulation was combined with some task familiarity. This facilitation was indeed not found when stimulation was administered in the first 'unfamiliar' session.

5.3 Effect of tDCS on working memory

On each testing day, all participants performed the n-back two times: immediately before and after receiving tDCS. Mean reaction times on the n-back task were not different between healthy younger and older adults, and across testing sessions. In addition, RT were shown to be unaffected by the application of offline tDCS.

The proportion of correct responses and number or omission/commission errors differed significantly across sessions, revealing a performance that was consistently better the second time that they performed the task on each day. This is common in studies that used the n-back task, which showed that this (difficult) working memory task is considerably affected by learning (Soveri, Antfolk, Karlsson, Salo, & Laine, 2017).

Generally speaking, it seemed that the more participants practiced the task, the higher their accuracy was. This improvement was found not only within one experimental session, comparing pre- and post-stimulation evaluations, but also across sessions, with better performance on day 2. These effects were more pronounced in older adults.

Older adults also made significantly more commission errors on this task, as compared to younger participants. Brain stimulation did significantly affect the number of commission errors. In parallel with what already described for the vigilance task, this effect seemed to interact with treatment order: participants who received sham in the first session, made fewer false alarms in the second real session, whereas the number of false alarms was stable across sessions for participants who received real followed by sham. In contrast to the effects of tDCS described for the vigilance task, brain stimulation did not significantly affect the proportion of correct responses and omissions made on the task. This difference may lie in the online/offline tDCS distinction, which has been suggested to produce effects mediated by different neural mechanisms: online-related effects depend on membrane depolarisation affected by ion-blocking substances, whereas offline-related effects involve the additional participation of NMDA receptor and therefore a LTP-like mechanism (Stagg & Nitsche, 2011).

6 Limitations and future work

The ultimate aim of the studies presented in this thesis was to examine the effect of tDCS in patients, and therefore the design was focussed around patients rather than healthy participants. In particular, the experimental paradigm was selected for its ability to detect a vigilance decrement in stroke patients (Malhotra et al., 2009). The chosen sample (healthy individuals) performed the task at very high accuracy levels and did not show any decrement in target detection over time. Such a high level of task performance may have led to a ceiling effect when evaluating the effect of tDCS on vigilance level (effect sizes were indeed indicative of small-medium effects) and the vigilance decrement.

Available studies into the effects of age on vigilance have produced inconsistent results, possibly due to the different tasks and the selection criteria (Davies & Parasuraman, 1982; Rogers, 2000). For instance, the mean age of my participants was 65 years old, and some studies have suggested that deficits in sustained attention begin to appear only in adults over the age of 70 (Filley & Cullum, 1994).

Another possibility is that performance at ceiling may have made it harder to fully appreciate the effect of tDCS on the vigilance decrement, which was not apparent in healthy adults. Following up on these findings, future studies focusing on the age-related vigilance decrement may utilise longer and/or more demanding paradigms to elicit a decrement in healthy individuals. Previous studies reported that prefrontal tDCS offsets the vigilance decrement after prolonged time-on-task (McIntire et al., 2017; Nelson et al., 2014), with a recent study asking participants to sustain attention for 80 minutes without any breaks (Reteig, van den Brink, Prinssen, Cohen, & Slagter, 2019). In alternative to lengthening the task, one could manipulate salience or increase the memory load, e.g., using a greater number of locations for stimuli to appear. Indeed, Caggiano and Parasuraman identified a correlation between vigilance decrement and working memory load, concluding that susceptibility to decrement is higher when the task involves a heavier memory load (Caggiano & Parasuraman, 2004). The task that was used here did not rely heavily on a memory load.

Despite the absence of any time-related change in performance, a consistent effect of tDCS on overall performance was observed, and this interacted with treatment order. In order to disentangle the two, a control experiment with participants undergoing two sham sessions could be carried out. Also, different parallel version of the vigilance task, with different sets of letters displayed at different locations on the screen, could be developed to minimise any learning effect. The use of different versions would have to be piloted in stroke patients for feasibility.

It would also be interesting to examine the duration of the effect of tDCS seen here.

In the future, studies could also investigate whether the repetitive application of tDCS over consecutive days can induce long lasting effects which generalizes to other measures of vigilant attention, and more ecological tasks.

7 Conclusions

The brain stimulation methodology used in this study can be safely used in healthy younger and ageing populations. In the current experiment, a superiority of brain stimulation as compared to sham treatment on vigilant attention levels emerged. This was most evident in older volunteers. It should be noted that, in this study, the effect of stimulation was found to affect different outcome measures, but for some of these outcome measures it was only observed in interaction with treatment order. Although some effect of practice may have contributed, practice alone could not account for the effects.

In the brain stimulation literature, researchers counterbalance treatment order assuming that this controls for any practice effects. Findings from this study suggest that counterbalancing real and sham stimulation between subjects may not be sufficient to control for the effect of practice. Considering that a recent study reported null effects of tDCS in a vigilant attention task performed by adults with ADHD, which the authors attributed to a significant practice effect between sessions that concealed potential stimulation effects (Jacoby & Lavidor, 2018), treatment order was systematically included in the analysis and was found to shape the effect of stimulation.

The precise targeting of the right dorsolateral prefrontal cortex used here may have had an effect on behaviour by modulating resting-state potential in neuron populations directly underneath the electrodes. One possibility is that tDCS may have influenced top-down attentional control, improving the ability to find targets among distractors (as in Brosnan et al., 2017). Since no vigilance decrement in accuracy levels was recorded for the sample of healthy participants, we cannot speculate on the possibility that tDCS may have directly affected vigilant attention,

which would be best tracked by changes in performance over time. The study of individuals who are more likely to exhibit a vigilance decrement with increasing time-on-task, such as individuals who have survived right-hemisphere stroke, may help shed light on this. This will be explored in Chapter V.

Another possible explanation for the results is that tDCS may have worked via boosting working memory. Indeed, the tDCS montage used here reduced the number of false alarms made on a verbal WM. However, this seems unlikely considering that WM demands for the vigilance task are minimal, as target identity remained static throughout the task and memory representation did not require any update, such that subjects needed to hold two spatial targets online for optimal performance. Moreover, impairment of spatial WM has been demonstrated to take place over periods of seconds (Mannan et al., 2005), whereas sensitivity in the current task did not decrease throughout the 15 minutes task. Future analysis of the card pairs task data will provide additional information that will be useful to clarify whether spatial working memory is affected by the application on offline tDCS.

Alternatively, as the DLPFC is part of large-scale fronto-parietal networks, prefrontal tDCS may have modulated activity in more posterior regions. The vigilance task incorporates a spatial component (i.e., letters are displayed at different locations), and required participants to continuously track the location of letters displayed on the screen. The application of tDCS may have facilitated access to spatial representations which are manipulated in the parietal cortex. One potential avenue to put this to test would be to have a sample of healthy participants perform a spatial task such as the Posner spatial cueing test, whilst receiving real and sham stimulation of the right DLPFC in a crossover design (Posner, 1980). This possibility will be partially explored in Chapter V by having a sample of stroke patients performing visuo-spatial tests before and after the application of brain stimulation. Also, whether the effect of brain stimulation could be attributable to a change in functional connectivity in attentional brain networks will be explored in Chapter VI, using an in-scanner application of brain stimulation.

The effects of the targeted application of prefrontal brain stimulation on vigilance and working memory diverged. A potential reason for this relates to the online/offline distinction: tDCS was applied online for the vigilance task and offline for the working memory tasks. More studies looking at the comparability of online and offline tDCS are needed to clarify potential differences in the induced effects. The tDCS montage chosen here was selected to target vigilant attention in right-hemispheric stroke patients. However, studies found that targeting the right DLPFC also leads to a tDCS-related improvement in the n-back task (e.g., Fregni et al., 2005). It is plausible that the significant learning effects found on the n-back may have partially masked some tDCS-induced changes.

Overall, this study provided insights into the understanding of the effect of prefrontal tDCS on attention and working memory measures in healthy adults. By emphasizing the close relationship between brain stimulation and treatment administration order, it also provided further support to the role of stimulation as a factor that may hasten learning and therefore enhance recovery. It also highlighted the importance of systematically examining stimulation effects in the context of learning and practice.

8 References

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CHAPTER V:
Efficacy of targeted tDCS application on attentional deficits following right-hemispheric stroke

1 Introduction

In the previous chapter, the targeted application of right prefrontal brain stimulation was shown to improve different measures of task performance in a sample of healthy adults. The potential of this approach in modulating attention in a clinical population, typically affected by severe attentional disturbances, will now be examined.

1.1 Vigilant attention following stroke

Right fronto-parietal networks, frequently directly damaged by a stroke, are critical in maintaining an alert state, as indicated by converging evidence (Posner & Petersen, 1990). For instance, in a meta-analysis of foci from five imaging experiments reporting activations that index arousal/vigilance in visual and auditory modalities, the majority of foci were observed in the right hemisphere, clustered around the temporo-parietal junction and prefrontal areas; these activations overlap the ventral attention network, which is typically lesioned in patients with neglect (Corbetta & Shulman, 2011).

Preliminary findings from the patient study described in this chapter have been published in abstract form. Olgiati, E., Violante, I.R., Li, L.M., Faraj, A., Sinclair, T., Crow, J., Wise, R., & Malhotra, P. (2019). Targeted brain stimulation to ameliorate vigilance in stroke: a combined tDCS-fMRI approach. *Brain Stimulation* 12(2):573.

In addition to the well-known impairment in spatial attention (see Chapter 1, Section 1.2.1), right-hemisphere stroke patients with neglect tend to be affected by vigilant attention problems, and struggle to maintain concentration regardless of the target location in space (see Chapter 1, Section 1.2.2). This vigilant attention impairment, common and persisting in this population, represents a potential rehabilitation target that has been underexplored (Robertson, 2001; Striemer, Ferber, & Danckert, 2013). Furthermore, it has been suggested that by targeting the non-lateralised component of the syndrome, the overall severity of neglect may be reduced (e.g., see Dalmaijer et al., 2018; Malhotra, Soto, Li, & Russell, 2013; Robertson, Tegner, Tham, Lo, & Nimmo-Smith, 1995).

1.2 Vigilance modulation by tDCS

Recent studies have suggested that vigilant attention can be boosted by prefrontal tDCS in healthy younger (Nelson, McKinley, Golob, Warm, & Parasuraman, 2014) and older individuals (Brosnan et al., 2018), as well as patients with traumatic brain injury (Kang, Kim, & Paik, 2012), opening up the possibility that this could be implemented for patients with focal lesions too. To date, there have been very few studies directly examining this.

In a proof-of-principle study, Kang and co-workers reported that offline tDCS improved accuracy on a go/no-go task in a sample of 10 stroke patients (Kang, Baek, Kim, & Paik, 2009). Active tDCS was found superior to sham at 1-hour and 3-hour post-stimulation timepoints, but with no detectable changes immediately after stimulation. However, this go/no-go task had minimal requirements in terms of working memory (respond to number '1' but not number '2') and vigilant attention (the task was very brief itself, comprising just 30 trials), precluding the possibility to examine the effects on the vigilance decrement.

In another study, Park and colleagues simultaneously targeted both dorsolateral prefrontal cortices in stroke survivors by using two anodal electrodes positioned over scalp coordinates F3 and F4, and two cathodal electrodes placed on the

nondominant left arm, one next to the other (Park, Koh, Choi, & Ko, 2013). Researchers applied 30 minutes of online real/sham tDCS daily, for 5 days a week until discharge, during a computer-assisted rehabilitation program targeting attention and memory. In a between-subjects design that included 11 patients, they reported an improvement in performance post- vs pre-treatment on a continuous performance test (CPT) in the tDCS group only. Despite these encouraging results, the choice of a between-groups design, with sub-acute patients affected by either right- or left-hemispheric lesions, is problematic. Critically, the two groups, who received either real or sham tDCS, started from a significantly different vigilant attention baseline, which is likely to represent a confound, potentially altering the results of the study.

The two studies mentioned above have started the process of exploring the potential of tDCS, alone and in combination with cognitive rehabilitation, to influence general attention levels in stroke. However, it should be noted that study inclusion/exclusion criteria were extremely broad, and patients with lesions anywhere in the brain were recruited - even though attention problems are typically persisting and disproportionately severe following right-hemispheric stroke (Bowen, McKenna, & Tallis, 1999; Denes, Semenza, Stoppa, & Lis, 1982; Ogden, 1985). In addition, no information regarding the presence and severity of other cognitive deficits was provided – this information might have been helpful in characterising the clinical population.

To my knowledge, a non-invasive brain stimulation approach has never been taken in order to modulate vigilant attention in a clinical population severely affected by persisting vigilant attention deficits, such as individuals affected by right-hemispheric brain damage. The present work represents an effort to develop this line of investigation.

2 Aim

The aim of the present study was to examine the efficacy of a precise application of prefrontal tDCS on vigilant attention in stroke survivors. The targeted area, the right dorsolateral prefrontal cortex, is a key area of the network specialised in maintaining an alert state. This region is typically spared by middle cerebral artery territory strokes and could therefore be susceptible to electrical modulation. Considering the interplay between lateralised and non-lateralised attention in the syndrome, the offline effect of tDCS on spatial attention was also examined as a secondary outcome.

3 Methods

The present study was designed to examine the efficacy of one dose of high-definition tDCS on vigilant attention in stroke. The methods utilised were identical to those described in Chapter IV, Section 3, with two exceptions concerning the population examined and the cognitive tasks performed before/after the application of brain stimulation (see Sections 3.1 and 3.4 below).

Briefly, a randomised, double-blind, sham-controlled, crossover design study was employed, consisting of two separate sessions (real and sham stimulation administered in counterbalanced order), at least 6 days apart. In each session, patients were engaged in a vigilant attention task whilst receiving 10 minutes of real or sham tDCS, in counterbalanced order. A short battery consisting of three standardised measures of the spatial bias was also performed offline.

3.1 Participants

Sample size for the study was arrived at from the power calculation described in Chapter IV, Section 3.1.1. Right-hemispheric stroke patients were recruited from those screened whilst on acute wards at Charing Cross Hospital. Before contacting patients to discuss participation in the study, I reviewed their clinical notes and

asked for a second opinion from a medically trained professional to ensure they satisfied the inclusion/exclusion criteria listed below.

Inclusion criteria:

- At least 3 months following stroke;
- Medically stable and able to attend cognitive testing and brain stimulation and possibly MRI;
- Evidence of attentional deficits in the acute stage.

Exclusion criteria:

- Inability to follow instructions or complete tasks pertaining to the experiment as a result of severe cognitive deficits or language barriers;
- Inability to give informed consent.

In addition, the inclusion/exclusion criteria previously discussed for healthy adults also applied (see Chapter IV, Section 3.1).

Clinical records for 117 patients who showed neglect whilst on the acute wards and who expressed interest in being involved in research were reviewed (for a thorough description of the cognitive screening methodology, see Chapter II, Section 2.2). 76 patients (65%) were excluded from participation because they did not meet tDCS inclusion and exclusion criteria (Table 5.1). A clinical history of seizures (and in one case an EEG suggestive of an epileptic focus) was the most common reason for exclusion in this sample (17% of the excluded individuals). The presence of a cardiac pacemaker (8%), incompatible with stimulation, was also relatively common in this population. 12% of the excluded patients were not invited to take part in research because at the time of recruitment they were living in residential home with full package of care or were bed-bound and unable to use a wheelchair. In 8% of the cases, after careful review of the medical notes, the baseline was not judged as neurologically intact as required by the study inclusion criteria, e.g. a strong suspect of a neurodegenerative conditions antecedent to the current stroke emerged.

Moreover, 5 patients whose lesion encroached the area that was going to be targeted by stimulation (i.e., the right DLPFC) were also excluded. In this cohort, another main reason for exclusion was history of a cranioplasty following post-stroke decompression. This was not originally included as a contraindication to brain stimulation, but these individuals were excluded to limit unnecessary risk: even if bone and glue only (i.e. not metal) were used in the procedure, the effect of brain stimulation through the skull when it has been surgically repaired and restored are still unknown.

Reasons for exclusion	**Individuals**
Metal in upper body	2
Seizures	13
Cardiac pacemaker	6
Severe cardiovascular history	3
Medically unstable	4
Cancer	2
Pain	1
Residential care/bed bound/24h package care	9
Anxiety/behaviour that challenge	4
Moved to country of origin	3
Deceased	7
Stroke not confirmed/bilateral stroke	4
Not independent at baseline	6
Lesion encroaching DLPFC	5
Cranioplasty/Craniectomy	7
TOTAL	76

Table 5.1: Main reasons for patient exclusion from the study
Numbers represent how many patients were screened on acute wards but not contacted for research for the reason listed in column 1.

41 patients with neglect were selected as potential candidates and were contacted via telephone and invited to take part to the study (Table 5.2). 9 of them did not respond to the invitation, or no valid contact detail was available. 9 patients did not agree to take part to research. For the 23 patients who agreed to take part, an appointment was then made to see them at the Clinical Imaging Facility, Hammersmith Hospital, at least 3 months post-stroke – patients had to be in their chronic stage of illness to minimise the confounds associated with spontaneous improvement over the course of participation. To increase representation of the

most severely affected individuals, wheelchair-accessible transportation was offered to patients and caregivers.

Response	**Individuals**
Not possible to contact/no response	9
No consent	9
Agreed to take part	23
Total	41

Table 5.2: Patient response to the invitation to take part in the present research
Numbers represent patients contacted for research.

One patient had to be excluded from any analysis on the basis of an incidental finding on the MRI scan performed as part of the study described in Chapter VI. Specifically, a large cyst was evident in the left frontal lobe. The final sample of tested individuals included 22 right-hemispheric stroke patients (mean age 65.545±11.722 years old, age range 44-85 years old, F=10, 20 right-handed) (Table 5.3). Handedness was assessed using a standard interview (Oldfield, 1971). Participants had normal or corrected-to-normal vision, with no history of previous neurological or psychiatric diseases. Due to unexpected data collection issues, two sessions had to be repeated. Specifically, two patients pressed the wrong key shortly into the task and responses were not recorded - in the following sessions, the other keys of the response box were masked to avoid confusion. No dropouts occurred for the patient group.

ID	Age	Sex	Handedness	Sleep Real	Sleep Sham	Motivation Real	Motivation Sham	Days between sessions
P1	77	M	R (100)	7	5	10	9.5	7
P2	69	F	L (-90)	7	7	9.9	9.9	7
P3	77	F	R (100)	6	5	6.9	5.8	14
P4	71	M	R (100)	6	6	9.8	9.9	7
P5	61	M	R (100)	8	7	9.9	9.9	18
P6	57	F	R (100)	9	11	9.6	10	12
P7	72	M	R (100)	7.5	6	9	NA	12
P8	54	F	R (100)	6	8	10	9.9	6
P9	85	F	R (100)	10	10	7.5	9.6	15
P10	63	M	R (100)	4	4	9.5	9.5	14
P11	46	F	L (-90)	6	9	9	8	7
P12	44	M	R (100)	8.5	9	9	8.3	72
P13	76	F	R (100)	8.5	7	7.4	5.7	56
P14	54	M	R (100)	7	5	8.7	8.6	10
P15	75	F	R (100)	8	6	10	5	104
P16	85	M	R (100)	7	8	9.9	7.4	14
P17	74	F	R (100)	9	12	9	6	11
P18	65	M	R (100)	8.5	8	9.3	7.4	90
P19	59	M	R (100)	7	7	10	10	10
P20	55	M	R (100)	8	8	8.8	9	10
P21	54	M	R (100)	7	7	6.1	5	18
P22	69	F	R (100)	7	7.5	5.1	6	11

Table 5.3. Demographics for chronic stroke patients who took part in the study
Sex: F=female, M=male. Handedness: R=right L=left (Oldfield). Sleep: hours of sleep the night before the session. Motivation: score on the VAS scale, with higher numbers indicating higher levels of motivation. NA=not available.

Clinical characteristics of the patient group are shown in Table 5.4. A neurological examination (see Chapter II, Section 2.2.1) was carried out with patients in the chronic stage of illness (>3 months following stroke). Ischemic stroke was the most common aetiology in this group. Median length of illness was 6 months (range 3-48 months). Patients who took part in the study presented across the full range of severity, and the sample included individuals who had been most severely affected by the cerebrovascular accident. 6 patients showed dense upper limb hemiparesis; 4 patients used a wheelchair and needed assistance of two to

transfer. Visual and tactile extinction were still common at the time of testing (respectively, 7 and 9 patients). Associated impairments such as anosognosia for hemiplegia (n=1, P3) and alien hand syndrome (n=1, P15) were relatively rare phenomena.

ID	Aetiology	Lesion location	Months post-stroke	M	SS left	SS both	V left	V both	Stars
P1	I	F-T-P	48	1/3	10 /10	0/10	10/10	10/10	24
P2	H	Th, BG	30	1/3	10/10	10/10	10/10	10/10	100
P3	I	F-T-P, BG	10	3/3	6/10	3/10	10/10	0/10	0
P4	I	IC	27	0/3	10/10	7/10	10/10	10/10	98
P5	H	T	3	0/3	10/10	10/10	10/10	10/10	47
P6	I	T-O, h, Th, CC	6	1/3	10 /10	10/10	10/10	9/10	28
P7	I	T-P	3	1/3	10/10	10/10	1/10	1/10	83
P8	I	CR, LN	5	2/3	10 /10	10/10	10/10	10/10	100
P9	I	BG, insula, fO	36	3/3	9/10	10/10	10/10	7/10	73
P10	I	T-P, insula, BG, fO	5	1/3	10/10	0/10	10/10	0/10	80
P11	I	F-T-P	48	1/3	8/10	0/10	10/10	5/10	62
P12	H	BG, CR	23	3/3	0/10	0/10	10/10	10/10	42
P13	I	F-T-P, CS	3	1/3	10/10	0/10	9/10	0/10	11
P14	H	T	5	0/3	10/10	10/10	10/10	8/10	98
P15	I	F-T, IC, LN	26	1/3	10/10	3/10	5/10	5/10	16
P16	I	P-O	3	3/3	10/10	10/10	0/10	0/10	22
P17	I	F-T-P, BG, insula	11	0/3	7/10	5/10	10/10	10/10	NA
P18	H	Th, IC	19	1/3	10/10	10/10	10/10	10/10	2
P19	I	T-P, insula	3	3/3	10/10	0/10	10/10	10/10	37
P20	H	Th	6	0/3	10 /10	10/10	10/10	10/10	24
P21	I	F, CR, insula, fO	3	1/3	10/10	10/10	10/10	10/10	79
P22	H	BG	4	3/3	0/10	10/10	10/10	10/10	4

Table 5.4: Clinical characteristics and performance on neurological examination for chronic stroke patients who took part in the study
All patients presented with a first right-hemispheric stroke which caused neglect manifestations in the acute stage. Aetiology: I=ischemic, H=haemorrhagic. Lesion location: F (frontal), T (temporal), P (parietal), O (occipital), BG (basal ganglia), IC (internal capsule), CS (centrum semiovale), LC (lentiform nucleus), Th (thalamus), h (hippocampus), fO (frontal operculum). M (motor upper limbs), 0=no deficit, 3=max deficit. V (visual upper quadrants) and SS (somatosensory upper limbs), 10/10= no deficit, 0/10=max deficit. Stars: proportion of identified targets on the star cancellation task performed on the ward in acute/post-acute stage of illness. NA=not available.

At time of recruitment on stroke wards, all patients had evidence of a lateralised deficit. By assessing performance on standardised measures of neglect carried out in the chronic stage, it was possible to evaluate whether patients were still showing signs of the pathological spatial bias characteristic of neglect. The clinical battery used here included two cancellation tasks and the line bisection test (Section 3.4.2). The same criteria used to diagnose cases of neglect acutely, as described in Chapter II, were adopted: neglect was deemed present if participants showed a lateralised deficit (i.e., a rightward bias on the line bisection task, asymmetry >5% on a cancellation task) on at least two tests, or on a single task in conjunction with a right-sided start on the cancellation tasks. An index of asymmetry for cancellation tasks (as opposed to the total number of targets found) was used as an indicator for the presence of a spatial bias. In fact, for many patients, performance on cancellation tasks is not at ceiling at re-assessment, but this may not necessarily indicate a problem with *lateralised* attention; it could be a by-product of the interaction between spatial and non-spatial attentional capacity (Husain & Rorden, 2003). Patients may interrupt their search prematurely, despite being given unlimited time, and show non-lateralised omissions. An example of this behaviour is shown in Figure 5.1.

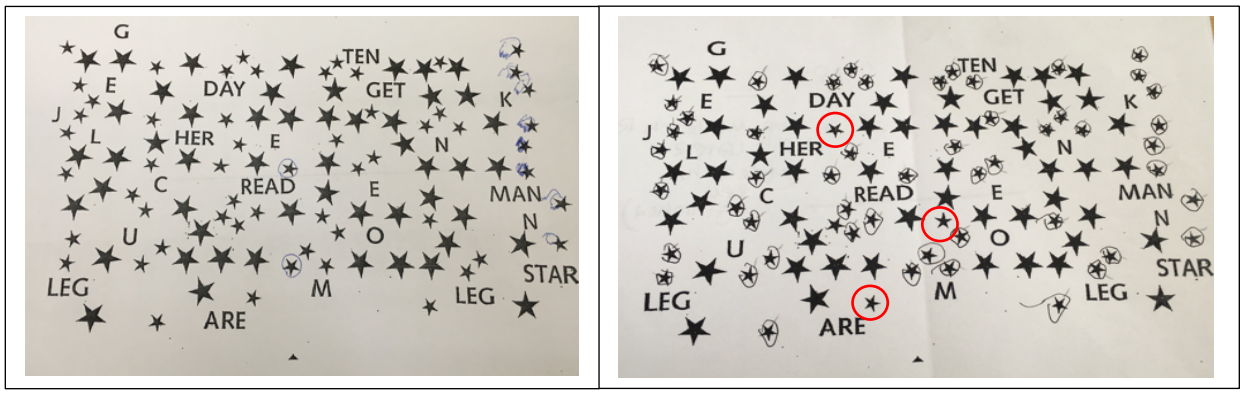


Figure 5.1: Star cancellation task performed in acute (left panel) and chronic stage (right panel) by a study patient
In the chronic stage, a patient made three omissions in the centre of the array (red circles), a pattern that is suggestive of non-lateralised attention deficit.

Using these criteria, 7 patients were found to have recovered from neglect. For the remaining 15 patients, neglect improved as compared to the acute stage; however, they still showed evidence of a persisting lateralised deficit at reassessment, of varying severity (Table 5.5).

Chronic assessment outcome	**Patients**
Recovered from lateralised deficit	7 (32%)
Persistent lateralised deficit	15 (68%)

Table 5.5: Recovery rates for lateralised manifestations of neglect

3.2 Experimental timeline

The experimental timeline for the study is similar to the one used for testing healthy participants and described in detail in Chapter IV, Section 3.2. The only difference concerns the tests that were performed before and after stimulation, as in this case clinical measures of neglect were employed.

Briefly, patients attended the laboratory twice, at least 6 days apart between sessions. For 10 patients, real tDCS was administered in the first session and a sham placebo tDCS in the second session. For the remaining 12 patients, the order of the treatment was reversed, in a double-blind study. At the beginning of each testing session, demographic data, sleep history and motivation levels were recorded. Head measurements were then taken, and electrodes were positioned over the target area. Patients were asked to perform clinical tests measuring lateralised attentional components, which were then repeated after stimulation. A computerised test for measuring non-lateralised vigilant attention was carried out whilst brain stimulation was delivered. The entire outline of each experimental session, which lasted for about 1.5 hours, is summarised in Figure 5.2.

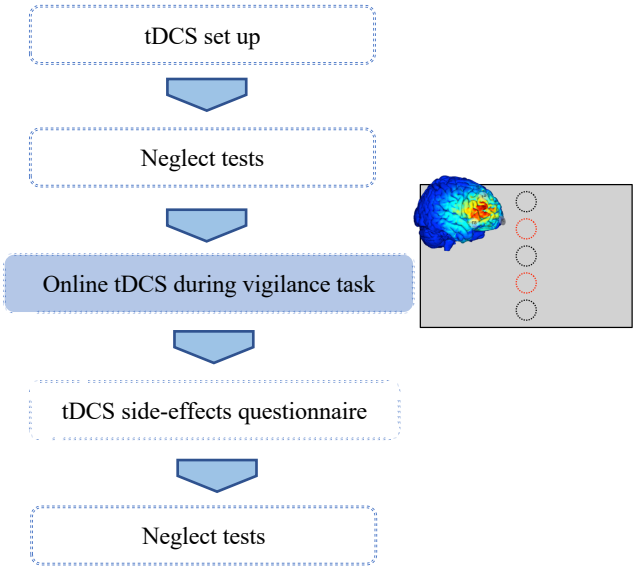


Figure 5.2: Outline of the experimental procedure
In two different sessions, patients received real or sham stimulation (in counterbalanced order) whilst they performed a vigilance task.

3.3 Brain stimulation

The set up for brain stimulation was identical to that described for healthy adults (Chapter IV, Section 3.2.1). Briefly, a precise targeted approach to stimulation was used, with anodal electrode over scalp coordinate F4 and two cathode electrodes over F8 and FP2, as defined by the EEG 10-20 system (Olgiati et al., 2019). This triangular montage allowed precise and optimal targeting of the right DLPFC, as confirmed by computational modelling (see Chapter III, Section 3.2).

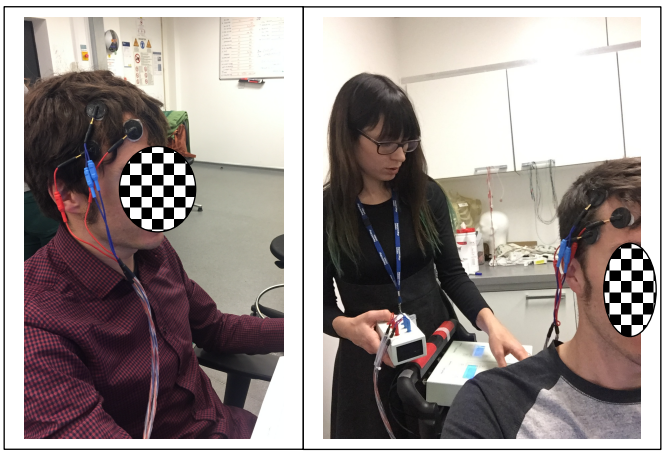


Figure 5.3: A study participant with electrodes on (left) and experimenter performing the tDCS habituation phase (right)

At the beginning of each session, electrodes were set up and a familiarisation phase was performed (Figure 5.3). In a familiarisation phase, brain stimulation was delivered at half intensity for 10 seconds, then at full intensity for 10 more seconds, to gently accustom patients to the sensations on the skin.

3.4 Tasks

The following cognitive tasks were performed during each session. During the first 10 minutes of the vigilance task, targeted brain stimulation was concurrently delivered.

The cognitive battery used to monitor spatial attention impairments characteristic of neglect was performed *offline*, i.e., before and after the application of tDCS.

3.4.1 Vigilance task

The vigilance task has been described in detail in Chapter IV, Section 3.3.1. Briefly, it required patients to continuously monitor a computer screen for 15 minutes, with targets displayed among distractors along the central vertical meridian of the screen. All patients completed a practice run-through of the task, until they reached 100% accuracy for 10 consecutive trials. For healthy adults, this required a maximum of 2 iterations (40 seconds) and for patients it required a maximum of 8 iterations (160 seconds). After the practice run, there was a brief break before the task, to avoid going immediately into the task. This familiarisation phase ensured that all participants understood the task, so as to minimise the possibility that a change in performance over time would reflect learning.

Before data collection was started, the task was test piloted in three right-hemisphere stroke patients to ensure tolerability (see Appendix VII). These pilot sessions also confirmed that the task was able to elicit a vigilance decrement in this clinical population. That is, stroke patients manifested a clear decline in performance over time.

3.4.2 Clinical battery

On each day, patients performed three clinical tests, in randomised order, before and after receiving brain stimulation. These tasks had already been used to screen patients in the acute stage, and have been described in detail in Chapter II, Section 2.2.2. Patients were given unlimited time to complete each test and had their performance video recorded.

The star cancellation task and the Mesulam cancellation task were administered (Figure 5.4 and Figure 5.5). Patients were asked to find and mark targets in a visual array whilst ignoring distractors. Cancellation tasks are normally considered the most sensitive single tasks for detecting the presence of a lateralised spatial bias (Azouvi et al., 2002; Ferber & Karnath, 2001).

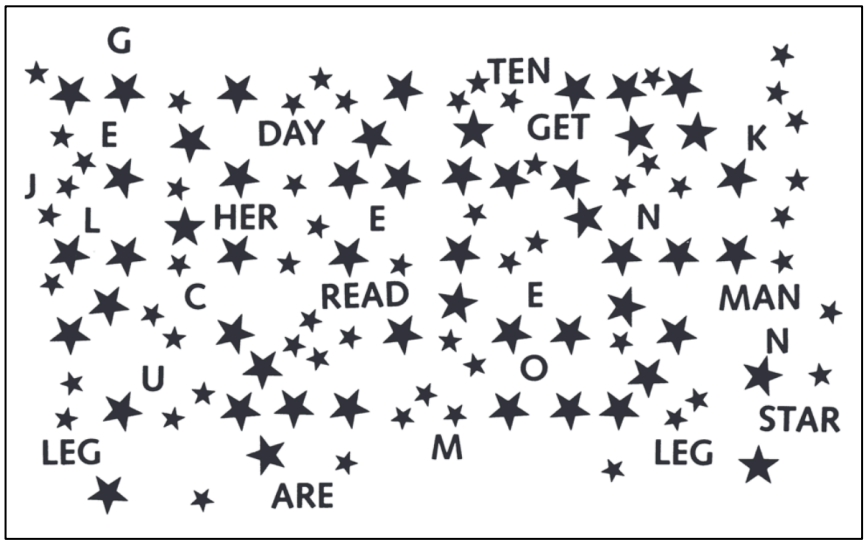


Figure 5.4: Star cancellation task (Wilson, Cockburn, & Halligan, 1987)
Patients are required to circle all small stars in the visual array.

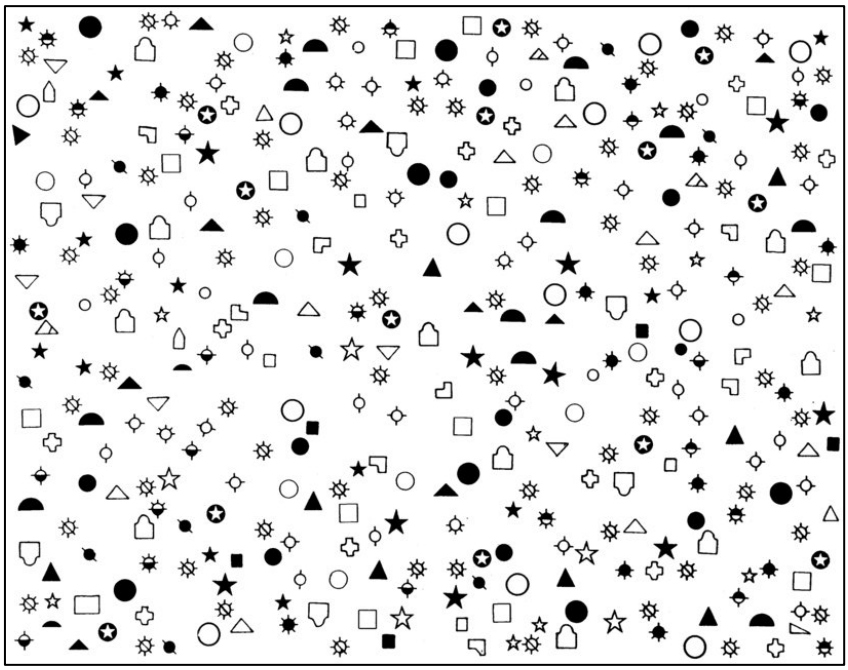


Figure 5.5: Mesulam cancellation task (Mesulam, 1985)
Patients are required to circle all target symbols in the visual array.

In addition, a line bisection task was used. Patients were presented with a set of six horizontal lines, centred on an A4 sheet (landscape orientation) of different lengths, and they had to find and mark the midpoint of each.

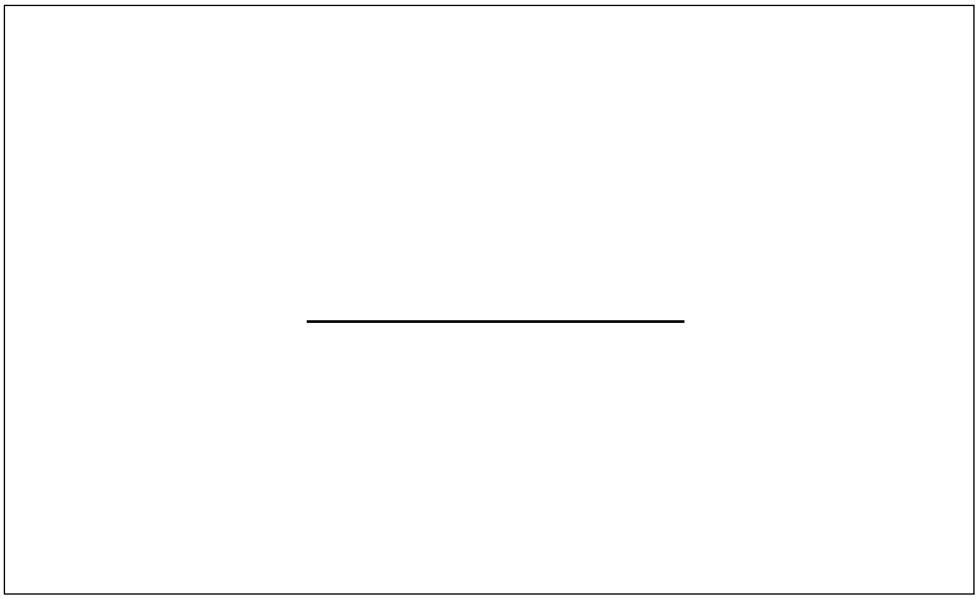


Figure 5.6: Line bisection task (Fortis et al., 2010)
Patients are shown a set of six lines (10, 15 and 25 cm long) and are asked to mark the midpoint of each.

3.5 Outcome measures

A side-effect questionnaire was administered by the examiner (Appendix V). Patients were asked to state whether they though they received any brain stimulation and to rate the intensity of any potential side-effect of stimulation

3.5.1 Vigilant attention task

My hypothesis was that tDCS would improve performance on the computerised attentional task at the group level. In addition, I hypothesised that some patients may respond, and other patients may not respond, to the application of tDCS. The same set of outcome measures discussed for healthy participants in Chapter IV, Section 3.4, were computed. Briefly, these were mean reaction times (RT), RT variability, errors (omission and commission errors), accuracy, target sensitivity (d') and response bias (C).

3.5.2 Lateralised deficits

The endpoints that were calculated for both cancellation tasks (as in Nurmi et al., 2018) were:

- total score, by calculating a proportion of targets effectively cancelled;
- asymmetry index, by calculating a difference between percentages of targets found on the right and on the left;
- processing speed, as indexed by time taken to complete the test;
- location of the first marking, which has been suggested to reflect initial ipsilateral orienting bias (Bonato, 2012; Nurmi et al., 2010; Samuelsson, Hjelmquist, Naver, & Blomstrand, 1996).

No time limit was set for these tasks. However, for total score and asymmetry index, the number of targets found within a time cut-off of 120 seconds was also derived. This information could serve as an indication of search efficiency.

For the line bisection task, percentages of rightward (positive values) and leftward (negative values) deviation from the centre were calculated by measuring, to the nearest millimetre, the subjective mark placed by each patient from the left end of

the line (see Chapter II, Section 2.2.2.2). Rightward deviations from the midpoint were given a positive value and those to the left a negative value.

3.6 Data analysis

Behavioural data was analysed using SPSS v26.0 (IBM, 2019). For each task, the shape of data distribution was assessed using the Kolmogorov-Smirnov test, as reported in Appendix VIII. Homogeneity of variance was assessed using Levene's test.

Paired sample t-tests and paired samples Wilcoxon signed-rank tests (two-tailed) were performed to compare sleep and motivation levels for the two experimental sessions.

For the vigilance task, accuracy and error scores were logarithmically transformed to address skewed data. All data were then analysed using a series of repeated measures analyses of variance (ANOVA). In all cases, effect sizes for significant effects (p value <0.05) were calculated using partial eta squared (η_p^2) and observed power was computed using alpha=.05. Bonferroni corrected post-hoc tests were used to explore significant effects.

To analyse measures of the lateralised bias, assumption-free statistical tests were employed due to the skewed distribution of the variables, which did not improve after logarithmic transformations. To evaluate the effect of tDCS on continuous variables (e.g., accuracy), Friedman's ANOVA (the non-parametric equivalent of a repeated measures ANOVA) was used. Pairwise comparisons for significant effects were performed using Bonferroni correction. To explore whether there was any relationship between categorical variables (e.g., the starting point on a cancellation task and the type of stimulation received), a contingency table was created, and the observed frequencies compared via a Pearson's chi-square test.

For all participants, including healthy adults who took part in the study described in Chapter IV, perception of stimulation was analysed via a series of Pearson's chi-square tests of independence. The intensity of each side-effect was analysed via a series of repeated measures ANOVA with Group (younger vs older vs stroke) as a between-subjects factor and Stimulation (real vs sham tDCS) as a within-subjects factor.

4 Results

Normality tests were performed on hours of sleep and motivation levels (Appendix VIII). The number of hours of sleep (normally distributed and analysed via a paired-sample t-test) was not significantly different at the two administration times (p>.05) (Table 5.6). Motivation, as assessed by the vertical VAS scale (Appendices III and IV), was not normally distributed and was accordingly analysed using the related-samples Wilcoxon signed rank test. Motivation levels were significantly different between real (median 9.3) and sham (median 8.6) sessions (p=.039). This difference seemed to be driven by the rating of one patient (P15), who reported to be twice as motivated on the day she received real as compared to sham stimulation, when her score was <.1.5 SD from the mean (Table 5.6).

	active tDCS	**sham tDCS**	**Related samples statistics**
Sleep	7.364±1.329	7.386±1.988	t(21)=-.072, p=.943
Motivation	8.829±1.387	8.114±1.837	z=-2.061, p=.039, r=-.318

Table 5.6: Mean (SD) amount of sleep (hours) and motivation score (VAS scale)
Sleep and motivation levels were both assessed at the beginning of each experimental session for all patients.

4.1 Vigilance task

4.1.1 Comparison to a control group

To confirm that patients were impaired on this task when compared with healthy age-matched volunteers, a one-way ANOVA with Group (older adults vs patients) as between-subjects factor was performed on RT and accuracy during sham stimulation. Both analyses revealed a main effect of Group, with patients performing slower [F(1,42)=25.544, p=.000] and less accurately [F(1,42)=22.456, p=.000] than healthy adults (older: mean RT 514.687, SEM 15.103 and mean accuracy 98.476, SEM 0.278; patients: mean RT 727.072, SEM 38.424 and mean accuracy 79.636, SEM 4.262) (Figure 5.7).

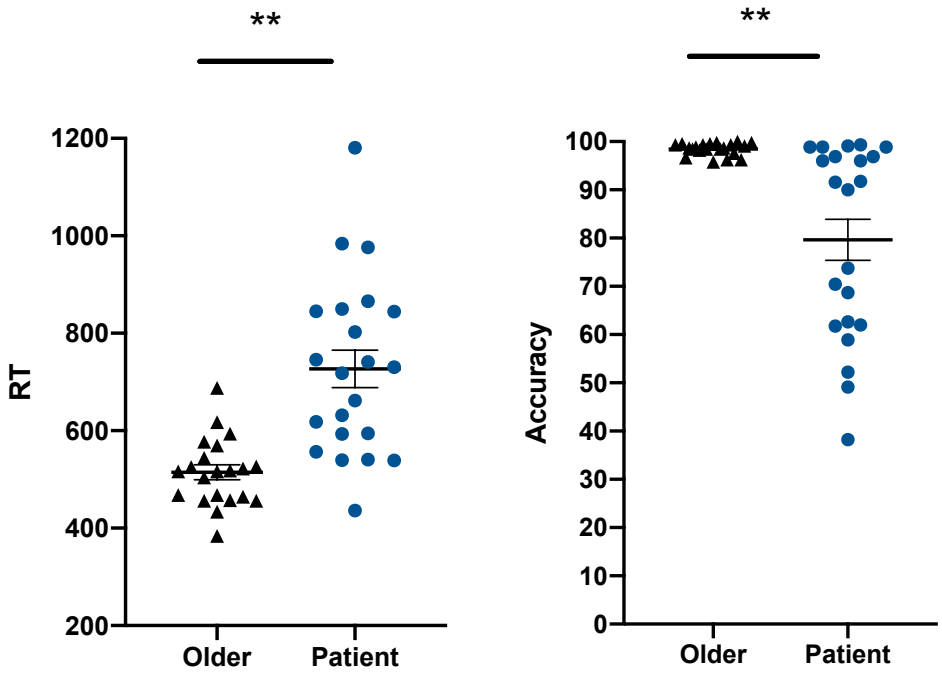


Figure 5.7: RT measured in milliseconds (left) and percentage of accuracy (right) on the vigilance task during sham stimulation for older adults and stroke patients
On average, stroke patients (blue dots) were significantly slower and less accurate on the vigilance task, as compared to healthy controls (black squares). RT with SEM are represented by black bars.

This analysis confirmed that, at the group level, patients performed less accurately on this task as compared to healthy participants. However, patient performance was heterogeneous. When looking at individual scores, not every patient

manifested a task performance deficit. As shown in Figure 5.8, 17 patients (77%, black dots) performed below the lower limit of the 95% confidence intervals for the mean of healthy older controls during sham stimulation (mean: 98.476, lower bound: 97.896, upper bound: 99.057). In other words, they manifested impaired performance on this task. 4 of them (18%) did not show any sign of a lateralised deficit on the clinical assessment, but showed a non-lateralised attention deficit on this task. 5 patients (23%, tangerine triangles) performed as accurately as controls: three of them did not show any sign of a lateralised deficit, whereas two manifested a mild spatial attention deficit on the line bisection task and on the Mesulam cancellation task.

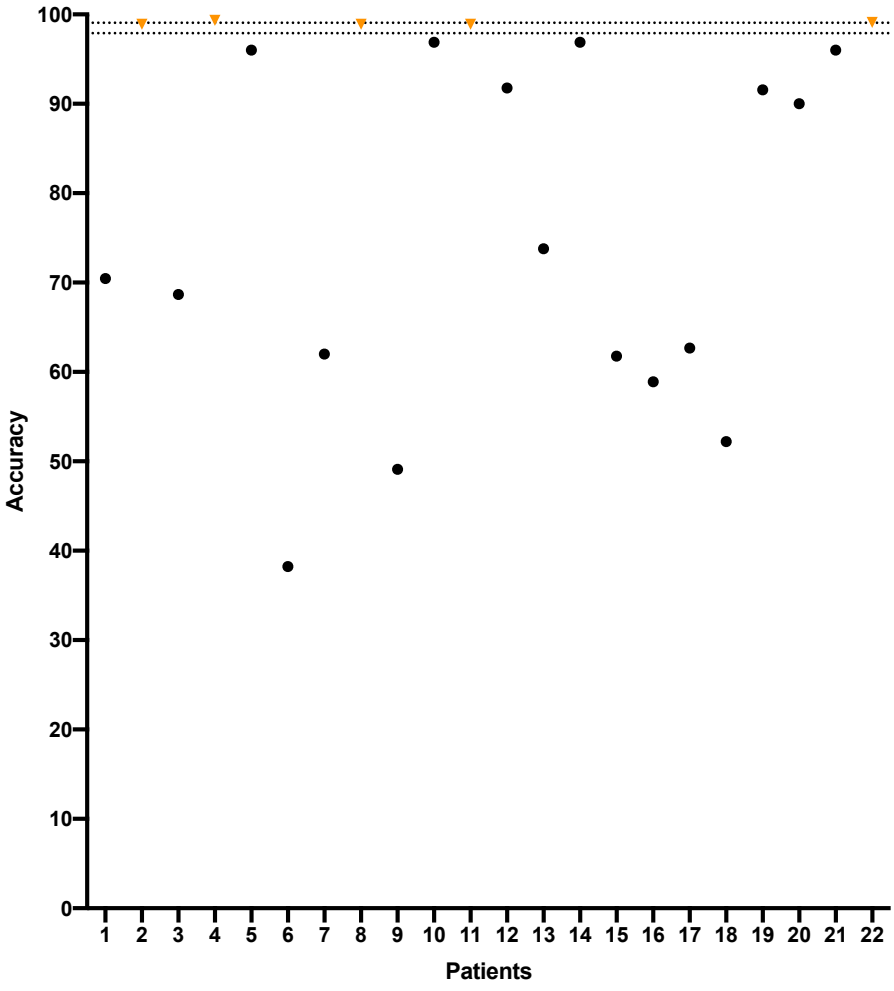


Figure 5.8: Accuracy on the vigilance task for patients
Dotted lines represent the 95% confidence intervals from a control population of healthy older adults. The vast majority of patients (black dots) performed less accurately than controls on this task.

4.1.2 Vigilance: overall performance

Data for the group of 22 patients who performed the vigilance task during real and sham stimulation (within-subjects design) were analysed. To explore the potential of the targeted application of prefrontal tDCS on task performance, a series of repeated measures analyses of variance (ANOVA) was performed, with Order (real-sham vs sham-real) as between-subjects factor and Stimulation (real vs sham) as within-subjects factor. Results for each dependent variable included in the model (mean RT, RT variability, total errors, omission and commission errors, accuracy, target sensitivity and response bias) are presented below.

4.1.2.1 Reaction times

Anticipations (RT <200ms) were removed. These represented a minority of responses (the 0.22% and the 0.44% of the total number of trials for two patients). The ANOVA of RT revealed a main effect of Order [$F(1,20)=7.294$, $p=.014$, $\eta_p^2=.267$, observed power=.729]: on average, patients in the group that received treatment in the sham-real order were slower to respond (mean 804.415, SEM 37.324), as compared to those who received real-sham (mean 627.632, SEM 23.841).

The main effect of Stimulation [$F(1,20)=.082$, $p=.778$] and the interaction between Stimulation x Order [$F(1,20)=2.168$, $p=.156$] did not reach statistical significance.

4.1.2.2 RT variability

The two-way ANOVA conducted on coefficients of RT variability did not reveal any significant effects, with main effects and interaction below statistical significance level [all p values >.05].

4.1.2.3 Total errors

Patients made a mean of 86.205 (SEM 13.340) errors on the task. The repeated measures ANOVA of log transformed numbers of total errors showed a main effect of Order [$F(1,20)=5.100$, $p=.035$, $\eta_p^2=.203$, observed power=.575], with participants in the real-then-sham group making fewer errors (mean 36.7, SEM

17.308) than patients in the sham-then-real treatment group (mean 127.458, SEM 26.76).

The main effect of Stimulation [F(1,20)=3.920, p=.062] and the interaction between Stimulation x Order [F(1,20)=.530, p=.475] did not reach statistical significance.

4.1.2.4 Omissions

60% of errors were omissions (i.e., missed target stimuli) and 40% were commission errors (where participants responded to a non-target). The ANOVA of number of omissions showed a significant main effect of Stimulation [F(1,20)=4.608, p=.044, η_p^2=.187, observed power=.533], with fewer omissions made during real (1069 in total, mean 48.951, SEM 12.167) as compared to sham (1204 in total, mean 54.727, SEM 12.1927) stimulation by patients (Figure 5.9).

The main effect of Order [F(1,20)=4.123, p=.056] and the interaction between Stimulation x Order [F(1,20)=.356, p=.558] were not statistically significant.

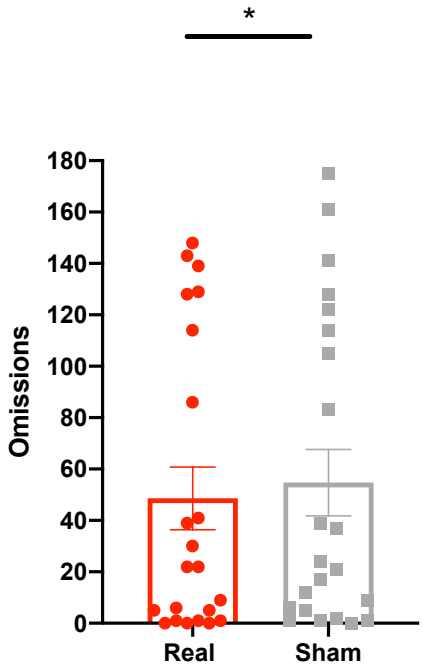


Figure 5.9: Number of omissions made during real and sham tDCS by patients
Patients missed fewer targets when they performed the task whilst receiving real, as compared to sham tDCS.

4.1.2.5 Commission errors

The repeated measures ANOVA of log transformed number of false alarms made on the task did not show any significant main effects or interaction [all p values >.05].

4.1.2.6 Accuracy

Accuracy was computed as a percentage of correct responses, incorporating hits and correct rejections. Stroke patients performed the vigilance task, on average, at an accuracy level of 80.84±19.45%. Reverse scored log transformed data for proportion of correct responses analysed via the two-way repeated measures ANOVA revealed a main effect of Order [$F(1,20)=5.379$, $p=.031$, $\eta_p^2=.212$, observed power=.598], with patients who received real tDCS first generally performing at higher accuracy levels (mean 91.844, SEM 2.662) as compared to those who received sham in the first session (mean 71.676, SEM 4.148).

No significant main effect of Stimulation [$F(1,20)=3.728$, $p=.068$] or interaction between Stimulation x Order [$F(1,20)=.637$, $p=.434$] emerged.

4.1.2.7 Target sensitivity (d')

The ANOVA of the ability to discriminate signal from noise revealed a main effect of Order [$F(1,20)=5.586$, $p=.028$, $\eta_p^2=.218$, observed power=.614], with patients in the real-sham group performing at higher sensitivity levels (mean 3.502, SEM 0.330) as compared to those who underwent treatment in the opposite order (mean 1.724, SEM 0.404).

The main effect of Stimulation was close to significance [$F(1,20)=4.229$, $p=.053$, $\eta_p^2=.175$, observed power=.499]: patients performed at a discriminability level of 2.716 (SEM 0.430) during real tDCS and of 2.348 (SEM 0.414) during sham tDCS (Figure 5.10).

The interaction between Stimulation x Order was not significant [F(1,20)=1.740, p=.202].

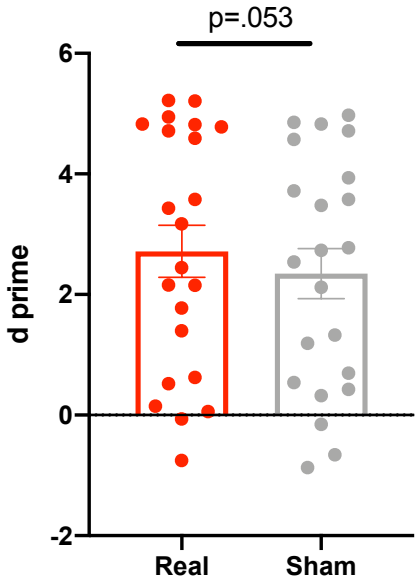


Figure 5.10: Target sensitivity (d') during real and sham stimulation in patients
There is a suggestion that patients were more able to discriminate targets from distractors when real tDCS was administered, as compared to sham.

4.1.2.8 Response bias (C)

A paired t-test examining criterion (C) during real and sham tDCS revealed no significant effects of stimulation on the strategy used to perform the task [t(21)=-.805, p=.430]. On average, the criterion was located -0.42 standard deviations away from the neutral point both during real and sham stimulation. This ruled out a possible effect of brain stimulation on the strategy used by patients to navigate the task.

4.1.3 Individual trajectories in response to tDCS

At the group level, the targeted application of prefrontal stimulation significantly reduced the number of missed targets on this task, improving target detection in the patient group. However, considering the well-known heterogeneity of this patient population (Stone, Halligan, Marshall, & Greenwood, 1998) and the inter-individual variability in response to tDCS shown by many studies (Wiethoff,

Hamada, & Rothwell, 2014), each patient trajectory was inspected separately (Figure 5.11).

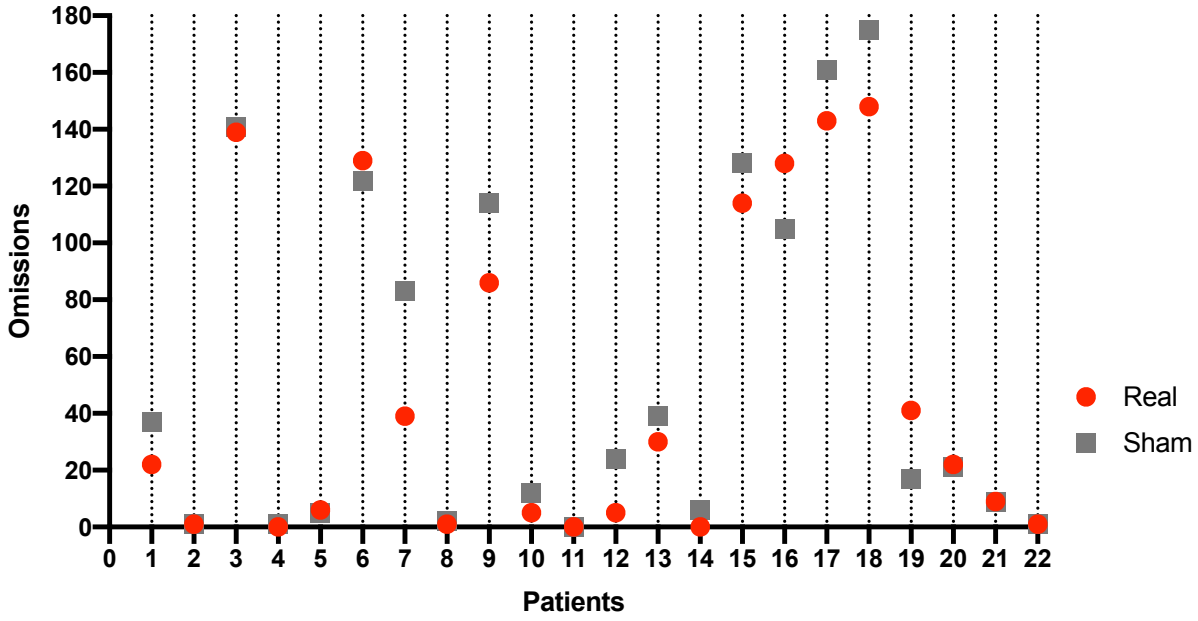


Figure 5.11: Task omissions during real and sham stimulation in patients
Patients showed different individual trajectories in responses to tDCS

It is possible to appreciate that patients showed different individual response to the targeted application of prefrontal tDCS. By calculating a difference between the number of omissions made on the vigilance task during real and sham stimulation, patients were classified into responders and non-responders (Table 5.7).

tDCS responsiveness	n(%)	Patient ID
Responders	13(59%)	1, 3, 4, 7, 8, 9, 10, 12, 13, 14, 15, 17, 18
Non-responders	5(23%)	5, 6, 16, 19, 20
Cannot be demonstrated	4(18%)	2, 11, 21, 22

Table 5.7: Summary of patient response to tDCS
Responders were patients who did improve on the real tDCS compared to the sham tDCS condition; non-responders were patients who got worse on the real tDCS compared to the sham tDCS condition; for a small proportion of patients, no difference in omissions was observed during real and sham tDCS.

The majority of patients were identified as responders, with fewer omissions made during brain stimulation as compared to sham tDCS. No clear effect could be demonstrated for 18% of patients, whose omission count remained stable across sessions - three out of four of them received real stimulation as first treatment. Another 22% of the patients omitted more targets during real tDCS, as compared to sham. This variability in response to tDCS is very common in tDCS studies, but poorly understood (Wiethoff et al., 2014). It would be critical to examine whether behavioural response to tDCS can be predicted and whether responders can potentially be differentiated from non-responders, for instance using neuroimaging. Anatomical and functional neural correlates of tDCS responsiveness in this experiment are analysed and discussed in Chapter VI.

4.1.4 Vigilance: decremental performance

Vigilance is best described as the ability to keep paying attention over time. According to some researchers, the best way to capture this is to look at what is called the vigilance decrement, i.e., any decline in performance with increasing time-on-task. To investigate whether the facilitating effect of stimulation extended to the vigilance decrement, I went on to analyse changes in RT, number of omissions made and target sensitivity across three time-epochs, each lasting for 5 minutes. Note that stimulation was on during the first two epochs, but off for the last five minutes of the task.

A series of ANOVA with Stimulation (Real vs Sham) and Epoch (1 vs 2 vs 3) as within-subjects factors were conducted on the sample of 22 patients.

4.1.4.1 RT

Mean RT for this analysis were not available for one patient, who did not make any button press in the last few minutes of the task; all other indicators of performance were nevertheless derived and analysed. The repeated measures ANOVA of RT did not show any significant main effects or interaction (p values >.05).

4.1.4.2 Omissions

The ANOVA of log-transformed number of omissions made on the task revealed a main effect of Stimulation [$F(1,21)=5.723$, $p=.026$, $\eta_p^2=.214$, observed power=.626], indicating that patients made fewer omissions across epochs during real as compared to sham stimulation.

A main effect of Epoch [$F(2,42)=9.873$ $p=.000$, $\eta_p^2=.320$, observed power=.977] also emerged, with patients omitting more targets in epoch 2 as compared to epoch 1 ($p=.004$), and in epoch 3 as compared to epoch 1 ($p=.007$) (Figure 5.12). A similar number of omissions were made in epoch 3 and 2 ($p=1.000$) when performance remained stable – note that in the real tDCS condition, tDCS was switched off for the third epoch.

The interaction between Stimulation x Epoch was not statistically significant [$F(2,42)=.536$, $p=.589$].

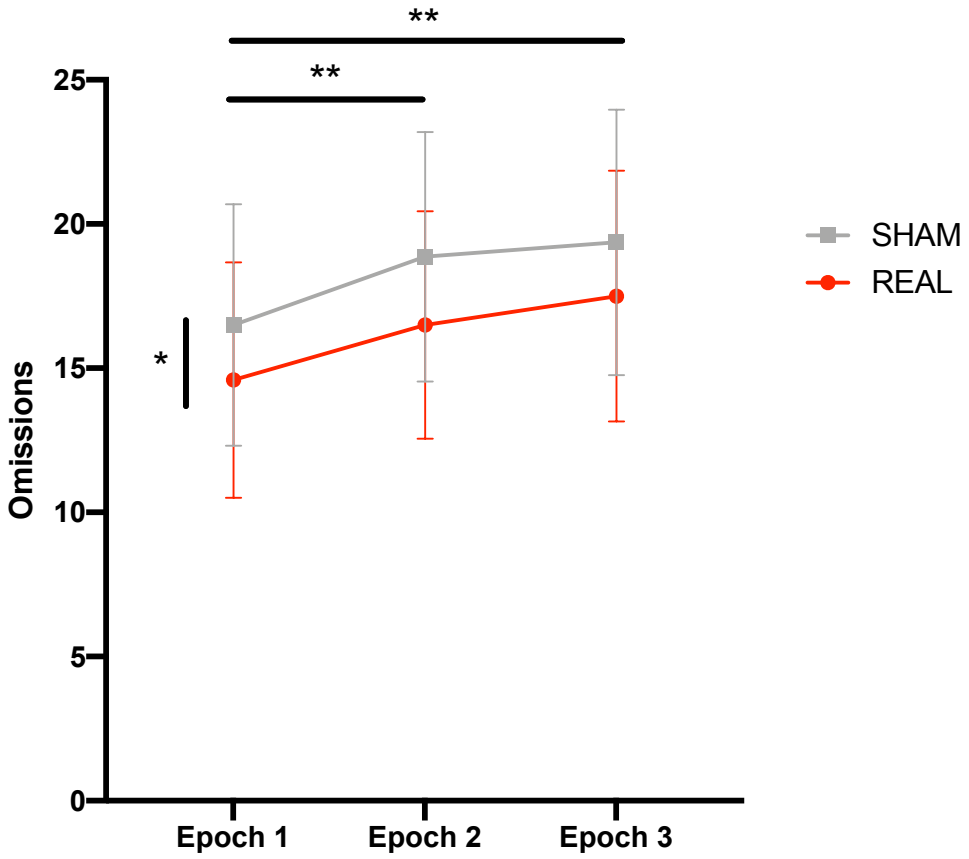


Figure 5.12: Omissions made by the patient group across time epochs

4.1.4.3 Target sensitivity (d')

The ANOVA examining d prime revealed a main effect of Epoch [F(2,42)=7.500, p=.002, η_p^2=.263, observed power=.927]: patients' ability to discriminate targets from distractors worsened between epoch 1 and 2 (p=.031), and between epoch 1 and 3 (p=.020), but was comparable between epoch 2 and 3 (p=.1000) (Figure 5.13).

The main effect of Stimulation [F(1,21)=3.701, p=.068] and the interaction between Stimulation x Epoch [(2,42)=.530, p=.593] did not emerge as statistically significant.

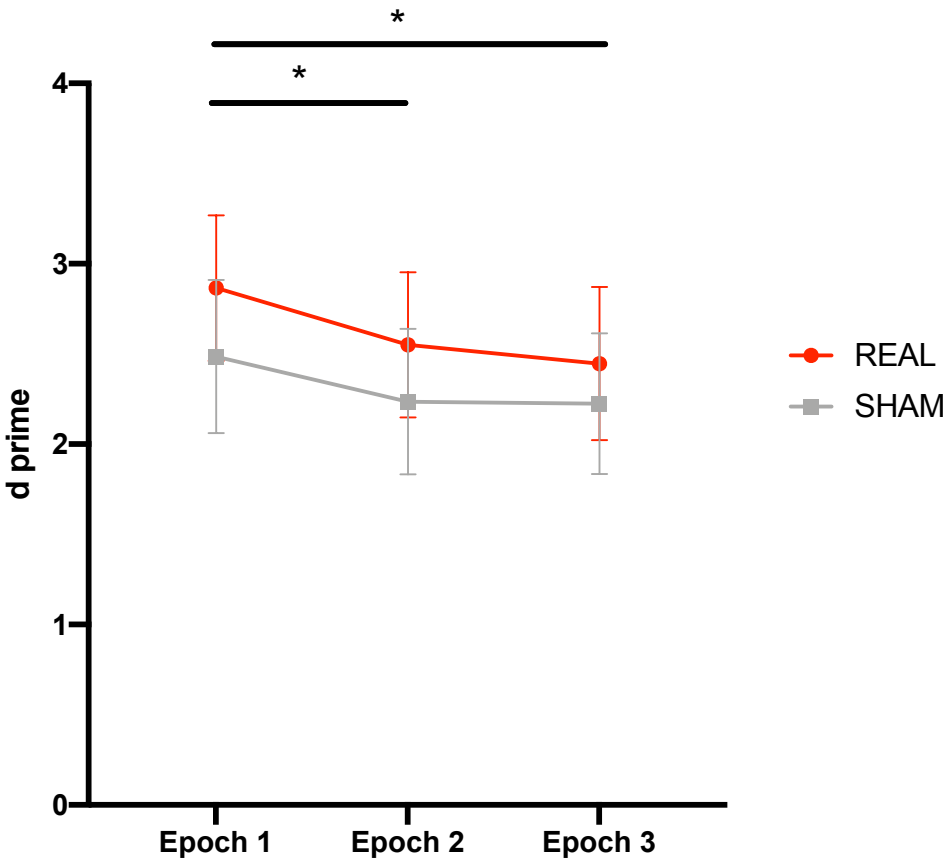


Figure 5.13: d prime across epochs for the patient group

To summarise the results of the vigilance decrement analysis, patients showed a clear decline in performance with increasing time-on-task. This manifested in an increase in omissions and a decrease in sensitivity with increasing time-on-task.

Specifically, patients omitted more targets in the central five minutes of the task, as compared to the first minutes. Performance in the last five minutes remained stable. Brain stimulation significantly improved performance, by reducing the number of missed targets across epochs.

4.1.5 Effect of tDCS on vigilance decrement

In order to explore whether the response to tDCS exhibited by a subset of patients was associated with a change in performance over time, the same analysis on vigilance decrement described in Section 4.1.4 was restricted to the 14 tDCS-responders (i.e., patients who missed fewer targets during real as compared to sham tDCS).

4.1.5.1 Omissions

The ANOVA of omissions showed a main effect of Stimulation [$F(1,13)=14.175$, $p=.002$, $\eta_p^2=.552$, observed power=.935], in keeping with the fact that this analysis was confined to tDCS responders only.

A significant main effect of Epoch [$F(2,26)=4.299$ $p=.024$, $\eta_p^2=.249$, observed power=.697] was also revealed, suggesting a change in omission rates with increasing time-on-task. However, after correcting for multiple comparisons, no difference between epochs was found significant (all p values >.05).

The interaction between Stimulation x Epoch was not statistically significant [$F(2,26)=.142$, $p=.868$].

A slope of the function fitting the data was calculated for number of omissions made on the task during real and sham tDCS. A paired t-test was then performed comparing slopes for real and sham tDCS, which showed that the slopes were comparable [$t(13)=-.198$, $p=.846$]. In other words, the steepness of the attentional decline was similar. When examining the intercept, however, a significant

difference emerged [t(13)=-2.327, p=.037], with patients performing at a higher levels throughout the task in the real vs sham stimulation condition (Figure 5.14).

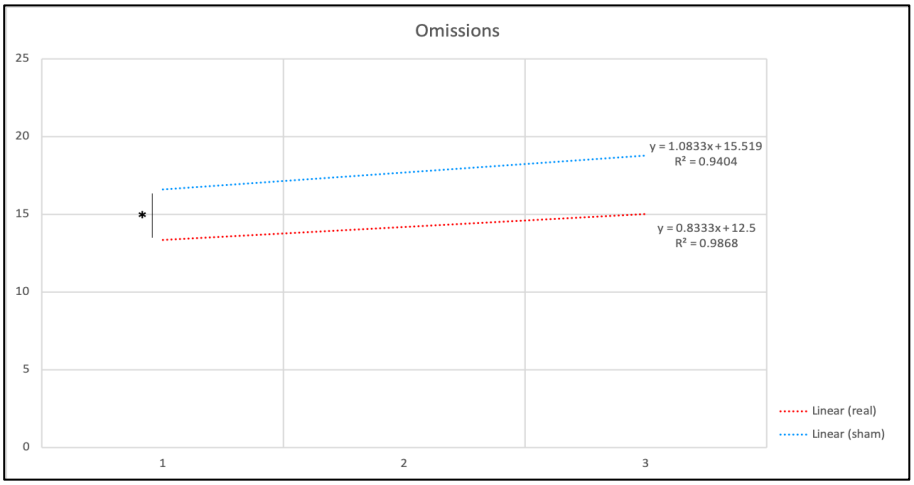


Figure 5.14: Functions for omissions per epoch (1, 2 and 3) made by responders during real and sham tDCS
The intercept for the two slopes was significantly different between real (+12.5) and sham tDCS (+15.5), with fewer omissions made throughout the task during brain stimulation.

4.1.5.2 Target sensitivity (d')

The ANOVA of target sensitivity showed a main effect of Stimulation [$F(1,13)=6.591$, $p=.023$, $\eta_p^2=.336$, observed power=.661].

A significant main effect of Epoch [$F(2,26)=3.743$ $p=.037$, $\eta_p^2=.224$, observed power=.632] was also revealed, indicating a difference in performance across epochs in patients. After correcting for multiple comparisons, no difference between epochs was found significant (all p values >.05).

The interaction between Stimulation x Epoch was not statistically significant [$F(2,26)=1.198$, $p=.318$].

Slope analysis showed that the curves were not significantly different [$t(13)=-1.200$, $p=.252$]. As described before for omissions, the intercept was found to be different for the two conditions [$t(13)=3.268$, $p=.006$] (Figure 5.15).

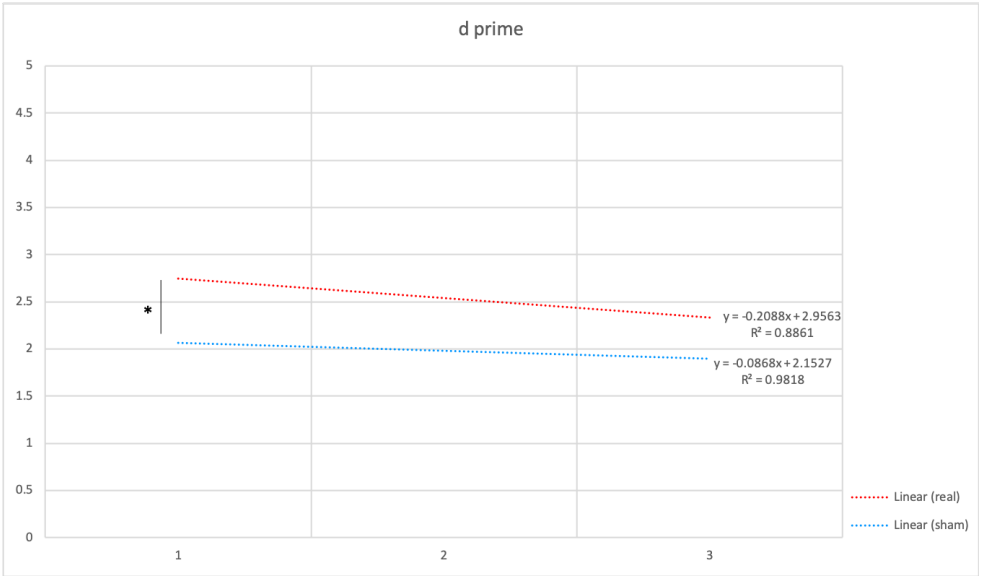


Figure 5.15: Functions for target sensitivity per epoch (1, 2 and 3) made by responders during real and sham tDCS
The intercept for the two slopes was significantly different between real (+2.96) and sham tDCS (+2.15), with higher sensitivity levels throughout the task during brain stimulation.

In sum, tDCS response did not manifest as a change in performance slope. Instead, brain stimulation had an effect throughout the task without affecting the shape of the slope.

4.2 Lateralised deficits

Patients underwent four testing sessions examining lateralised deficits of neglect, i.e., before and after real and sham tDCS. Performance was compared between sessions.

4.2.1 Star cancellation task

On average, patients performed the star cancellation task at high accuracy levels already at the pre-stimulation evaluation (mean 88%, SEM 2.742).

4.2.1.1 Total score

The total score on the star cancellation task did not significantly differ across testing sessions [$\chi 2(3)=4.241$, $p=.237$].

Even when a cut-off time limit of 120 seconds was imposed in calculating the score, no significant changes between sessions emerged [$\chi2(3)=4.717$, p=.194].

4.2.1.2 Asymmetry index

The number of targets found on the left vs on the right was not significantly different across sessions [$\chi2(3)= 3.082$, p=.379]. Similarly, when the 120s cut-off was applied, no significant difference emerged [$\chi2(3)=2.753$, p=.431].

4.2.1.3 Processing speed

On average, patients took 133.068±142.241 seconds (range: 34-900) to complete the task. The time spent searching for targets did not significantly differ across sessions [$\chi2(3)=6.205$, p=.102].

4.2.2 Mesulam cancellation

4.2.2.1 Total score

The total score on the Mesulam cancellation task was not significantly different across sessions [$\chi2(3)=6.369$, p=.095].

When a cut-off time limit of 120 seconds was imposed in calculating the score, a significant difference across sessions emerged [$\chi2(3)=9.827$, p=.020]. After correcting for multiple comparison, only the contrast between targets found in the pre-real tDCS (mean 65%, SEM 6%) as compared to the post-real tDCS session (mean 71%, SEM 6%) was at significance level [p=.0560].

All other planned contrasts were not significant: no difference emerged between pre- and post-sham tDCS sessions, as well as between pre- and post-stimulation evaluations [all p values >.05].

4.2.2.2 Asymmetry index

The asymmetry in targets found on the left vs on the right was not significantly different across testing sessions [$\chi2(3)=3.162$, p=.367].

Likewise, when the 120s cut-off was applied, no significant difference emerged [$\chi 2(3)=1.340$, $p=.720$].

4.2.2.3 Processing speed

On average, patients took 185.702±122.477 seconds (range: 62-600) to complete the task. The time spent on the task was found to be significantly different across sessions [$\chi 2(3)=8.127$, $p=.043$]. However, after correcting for multiple comparisons, no significant difference survived [all p values >.05].

4.2.3 Line bisection task

On average, before any stimulation was applied, patients bisected the lines with a +1.409±11.903% rightward displacement from the true centre. The large standard deviation indicated the presence of positive and negative values, i.e., rightward and leftward displacements in the patient sample.

Friedman's two-way analysis of variance (ANOVA) by ranks was used to compare mean percentages of displacement across the four different evaluations: pre-real, post-real, pre-sham, post-sham. A statistically significant difference in performance between sessions emerged [$\chi 2(3)=8.250$, $p=.041$]. After Bonferroni post-hoc tests were carried out to compare for the 4 contrasts, no significant differences between sessions survived [all p values >.05].

5 tDCS blinding and side effects

Blinding success and perceived discomfort related to stimulation were assessed after each stimulation period using a questionnaire (Brunoni et al., 2011) (Appendix V). This was administered to stroke patients as well as healthy adults who took part in the study described in Chapter IV. All participants were asked to state whether they thought they received any stimulation or not, and to rate the intensity of 9 potential side-effects on a scale from 0 (absent) to 3 (severe). Data for 18 younger adults, 21 older adults and 22 stroke patients were analysed as described below.

5.1 Perception of stimulation

A first analysis aimed to determine whether real and sham targeted tDCS were undistinguishable when delivered in two different sessions, at least 6 days apart. To relate the two categorical variables, 'stimulation delivery' (yes vs no) and 'stimulation perception' (yes vs no), a series of Pearson's chi-square tests of independence (one per group) was performed (Figure 5.16).

In younger adults, the relation between these variables was significant [$\chi^2(1)=8.53$, p =.003]: 75% of younger participants were able to correctly identify the stimulation condition, i.e., real or sham tDCS. When questioned, they stated they had received stimulation on 62% of occasions, indicating a tendency to overreport stimulation.

For older adults, the association between delivery and perception of stimulation was significant [$\chi^2(1)=4.71$, p =.03]. The stimulation condition (real vs sham tDCS) was correctly identified 67% of the times, that is above chance. In 55% the cases, they stated they had received sham stimulation.

Perception of stimulation was at chance level for stroke patients [$\chi^2(1)=0.419$, p =.517]. Patients correctly recognised the stimulation condition 54% of the times, i.e., they were at chance level. Generally, patients showed a slight tendency to overreport having received stimulation, stating they received stimulation 68% of the times.

tDCS DELIVERY	tDCS PERCEPTION	
Younger	YES	NO
YES	14	2
NO	6	10

tDCS DELIVERY	tDCS PERCEPTION	
Older	YES	NO
YES	13	8
NO	6	15

tDCS DELIVERY	tDCS PERCEPTION	
Stroke	YES	NO
YES	16	6
NO	14	8

Figure 5.16: Awareness of stimulation for the three groups of participants
The contingency tables show, for each group, participants' awareness of the stimulation condition. In a crossover design, participants all received 10 minutes of real and sham tDCS during task performance in two different sessions, at least 6 days apart.

By comparing responses to the two treatments for each individual patient, I discovered that only 5 patients were able to correctly identify both the real and the sham tDCS conditions. In other words, 5 patients were able to tell real and sham tDCS apart. Two of them showed a response to the application of tDCS, whereas the other three patients did not show a response/showed a cost. It follows that the vast majority of responders (11 out of 13), who benefitted from the application of tDCS, were not able to disentangle real and sham tDCS.

5.2 **Intensity of tDCS side-effects**

Itchiness and tingling sensations, together with sleepiness and difficulty in concentrating, were the most severe side-effects reported by study participants. After receiving real tDCS, these subjective sensations were reported as contraindications with an intensity within the absent to mild range (respectively, 0.46/3, 0.41/3, 0.33/3).

A series of repeated measures ANOVA with Group (younger vs older vs stroke) as a between-subject factor, and Stimulation (real vs sham tDCS) as a within-subjects factor, was performed on the subjective intensity of each side-effect. The average severity of all subjective side-effects is shown in Table 5.8, together with the probability value for the main effect of Stimulation.

For tingling sensations, a main effect of Group emerged [$F(2,58)=6.802$, $p=.002$, $\eta_p^2=.190$] and was explored with Bonferroni corrected post-hoc comparisons: older adults perceived pins and needles-like sensations less than younger adults (younger: mean=0.889, older: mean 0.262) [p=.002]. Tingling was also rated as more intense following real as compared to sham tDCS [$F(1,58)=.9.153$, $p=.004$, $\eta_p^2=.136$]. The interaction between Group x Stimulation was not significant [p value >.05].

Side-effects	Active tDCS	Sham tDCS	p value
Tingling	+0.74	+0.43	p=.004
Itching	+0.46	+0.21	p=.024
Scalp pain	+0.1	+0.02	p=.005
Neck pain	+0.02	+0.00	p=.263
Headache	+0.02	+0.05	p=.438
Skin redness	+0.02	+0.00	p=.263
Sleepiness	+0.41	+0.40	p=.908
Difficulty to concentrate	+0.33	+0.53	p=.144
Acute mood change	+0.06	+0.03	p=.284

Table 5.8: Mean adverse effects reported by all subjects after real and sham tDCS
Numbers corresponds to the average severity of each subjective side-effect.
P values are derived from a series of repeated measures ANOVA.

Similarly, a main effect of Group emerged for the itching sensation [$F(2,58)=6.184$, $p=.004$, $\eta_p^2=.176$], with older adults perceiving this less than younger adults (younger: mean=0.639, older: mean 0.95) [p=.003]. Itching was reported as more severe following real tDCS as compared to sham tDCS across all groups [$F(1,58)=5.403$, $p=.024$, $\eta_p^2=.085$]. The interaction between Group x Stimulation was not statistically significant [p value >.05].

Scalp pain was reported at significantly different intensities by different groups [$F(2,58)=8.865$, $p=.000$, $\eta_p^2=.234$]: younger adults were the only group to report this sensation (mean +0.194) after real/sham tDCS, whereas older adults and stroke patients did not perceive any pain [p values <.05]. Scalp pain was reported to be more intense after real as compared to sham tDCS [$F(1,58)=8.338$, $p=.005$, $\eta_p^2=.126$], with a significant interaction Condition x Group [$F(2,58)=7.863$, $p=.001$, $\eta_p^2=.213$]: it was real tDCS that was reported as more painful as compared to sham tDCS by the younger group (mean +.333), as compared to the older (mean 0.000) and patient group (mean 0.000) [p=.001]. Also, younger adults reported scalp pain more intensely following the application of real tDCS as compared to sham tDCS

(respectively, +0.333 and +0.056) [p=.000]. All other pairwise comparisons were not statistically significant [all p values >.05].

Headache, neck pain, occasional redness at the site of the electrodes, mood change, sleepiness and difficulty to concentrate, all occurred at nearly the same rate after real or sham stimulation in all groups of participants (all p values >.05).

In sum, brain stimulation was well tolerated in this healthy and patient cohort, with mild-moderate side-effects and no dropouts. Analysis of reported side-effects showed that, on average, younger and older adults appeared to be able to distinguish between real and sham tDCS; however, stroke patients were at chance level. General discomfort was very low, and the intensity of all side effects was on average absent or mild. Tingling, itching and scalp pain were reported as more intense post-real as compared to sham tDCS, in particular by the younger group.

6 Discussion

The present study aimed at investigating whether a single dose (10 minutes) of tDCS, targeted to the right dorsolateral prefrontal cortex, has beneficial effects for individuals with attentional deficits following right-hemispheric stroke. The tDCS montage used in this work allowed precise targeting of the right-lateralised frontoparietal vigilant attention network. Damage to this network has repeatedly been demonstrated to be critical in the development of non-lateralised attentional deficits in right hemisphere stroke, and in the pathogenesis of neglect.

6.1 tDCS effect on non-lateralised attention deficit of neglect

A computerised paradigm was used to investigate the effect of tDCS on non-lateralised attention abilities in right-hemispheric stroke patients. Participants were asked to sustain attention during a 15 minutes spatial task, with targets and distractors sequentially displayed along the central vertical meridian of the screen.

Results indicate that tDCS, when compared to sham stimulation, led to a significant improvement in the total number of identified targets. In other words, at the group level, patients missed fewer targets when brain stimulation was administered concurrently with task. When target sensitivity, a statistic derived from signal detection theory, was computed, there was a suggestion that this index may follow the same pattern of improvement for the real as compared to the sham stimulation condition. The magnitude of the effect size for this index (d prime) was however smaller. This suggests that the relationship between the variables (d' and stimulation) was not as strong as that discussed for omissions. This may be because d prime is also sensitive to the number of commission errors, which appeared to be unaffected by stimulation. This may have potentially reduced the overall effect of stimulation on this index.

Accuracy rates were not found to be affected by the application of tDCS. This could relate to the way accuracy is computed in tasks where targets and non-targets are not present in equal numbers, as in the case of the vigilance task used in this study; i.e., targets have a 40% presentation rate and distractors have a 60% presentation rate. In this case, a participant not responding at all is assigned an accuracy of 60%, which may lead to the paradoxical result of a patient not responding having higher accuracy rates than other patients who responded but made mistakes.

In keeping with what discussed for healthy participants in Chapter V, no tDCS-induced modulation of reaction times emerged.

The analysis also revealed a main effect of the order group, with patients receiving the treatment in the order sham-then-real responding generally more slowly and less accurately than the group who received the same treatment but in reverse order. Although patients were randomly assigned to one of the two treatment orders, considering the relatively low numbers of patients, it is still possible that the two groups were not entirely matched in terms of attentional performance.

A decision was made to randomly assign patients to the two treatment groups (Real-then-Sham vs Sham-then-Real). They all received the treatment, only in different orders. A study design with a baseline session would have been helpful in balancing patient assignment to treatment arms. The possibility of incorporating a baseline session was considered in the design of the present study but was not implemented, because it would have increased the potential effect of task practice and, more importantly, increased demands on patients and dropout risks. It is however critical to highlight that the effect of order group was never found to interact with that of stimulation, in any outcome measure. This suggests that stimulation had an effect on performance in both groups (i.e., the group who performed the task, on average, at higher accuracy level as well as the group who performed at lower sensitivity levels).

There was no evidence supporting the notion that the beneficial effect of tDCS was mediated by a modulation of the vigilance decrement. Specifically, patients' performance deteriorated with increasing time-on-task; in other words, they showed a vigilance decrement. This decline in performance over time followed a similar pace for when the task was conducted whilst receiving real or sham stimulation. The tDCS montage used here did not seem to slow down or abolish the vigilance decrement, and patients got worse at the task over time regardless of stimulation. A similar result was arrived by another study that employed an incremental reward manipulation in healthy younger adults where reward was delivered on a trial by trial basis, increasing overall performance (i.e., higher accuracy and lower response variability) on a task requiring frequent responses, leaving the vigilance decrement time unaltered (Esterman, Reagan, Liu, Turner, & DeGutis, 2014). In a subsequent study, however, the researchers showed that the vigilance decrement could be attenuated when a reward was anticipated but withheld until the end of the 10-min run (Esterman et al., 2016). Further research directly examining the comparability of the continuous administration of rewards and brain stimulation might be helpful in clarifying these effects.

In this study, the effect of tDCS was found to last throughout the task, outlasting stimulation duration: its effect and superiority to sham stimulation persisted for at least five minutes after the stimulation was stopped. Although physiological effects induced by tDCS are still unclear, proof-of-principle studies examining the time-course of the effect have shown that conventional stimulation of motor areas lasting longer than nine minutes outlasts the stimulation duration, inducing after-effects on cortical excitability lasting up to 90 minutes (Nitsche et al., 2003; Nitsche & Paulus, 2001). In healthy individuals, the available evidence suggests that the principal effects of HD-tDCS fit those of conventional tDCS (Kuo et al., 2013). Interestingly, a study showed that the plastic changes induced by targeted tDCS created a more delayed peak at 30 minutes, and longer lasting after-effects for more than 2 hours after (anodal and cathodal) tDCS, as compared to conventional tDCS (Kuo et al., 2013). Future studying investigating the timing of the tDCS, probing the wearing-off of its effect at different time intervals post-stimulation are needed.

To summarise tDCS effects on non-lateralised attention, brain stimulation improved task performance in this patient group, with a significant effect that was evident on the number of omissions made on the task. It is worth noting that brain lesions for patients entering the study excluded the target area, namely the right DLPFC. This choice was deliberate and intended to maximise the chances to observe any effect of tDCS. By applying this exclusion criterion, individuals with less potential to benefit from the treatment were not enrolled. This approach may have allowed me to be in an ideal position to detect a main effect of stimulation at the group level.

The present findings could be interpreted as demonstrating a tDCS driven improvement in a non-lateralised component of neglect. In this cohort, a reduction in the number of missed targets on the attentional task was demonstrated when tDCS, as compared to sham, was administered concurrently to task. Nevertheless, no change in time-on-task performance could be established, which makes it

unlikely that tDCS may have exerted its effects by modulating vigilant attention in the strictest sense (i.e. relating to the vigilance decrement).

Another possibility is that tDCS may have boosted performance via increasing general alerting and arousal levels on a trial-by-trial basis, potentially via the same mechanism as motivational and pharmacological stimulations (Dalmaijer et al., 2018; Olgiati, Russell, Soto, & Malhotra, 2016). However, it should be noted that no change in RT was observed, which might be considered to be more in keeping with a modulation of arousal levels. Concurrent physiological recordings (e.g., skin conductance, pupillometry) were not acquired as part of this work, but could potentially help clarify whether the effect is arousal mediated.

Another possibility is that the effect of tDCS on task performance was mediated by a modulation in space representation, which is typically affected in this clinical population. The spatial component built into the task indeed demands a tight interplay between frontal areas and parietal regions, which contain spatial representations. The specific tDCS montage used in this study, targeting an area that is part of large-scale networks, may have facilitated fronto-parietal coupling, and influenced activity in posterior areas. Offline tDCS, however, did not seem to modulate the spatial bias, as measured using standard clinical tests for neglect (see Section 6.3 below). Potential ways to directly test the hypothesis that the tDCS montage used here worked by modulating spatial representation would be to compare these findings with those of tDCS on an attentional task that does not incorporate a spatial component. In addition, one could potentially explore the functional correlates of tDCS responsiveness in this clinical population. This would allow examining whether the effect is related to a modulation in networks that are important for space representation – this will be the subject of investigation of the imaging study presented in Chapter VI, Section 4.

As discussed in Chapter IV for healthy participants, a modulation in working memory could also account for the effect of brain stimulation. In fact, one of the major regions that shows task related activation in working memory paradigm

seems to be the DLPFC, which is the stimulation target in this study (Petton et al., 2019). The task that I used, in addition to testing for the (attentional) ability to coordinate and sustain goal-directed behaviour, included a WM component. The latter would involve, for each trial, comparing the letter on the screen with the template from memory, and support the decision to press/not press the button. The purpose for holding this information would be that of supporting goal-oriented behaviour. Given this close relationship between working memory and the executive component of attention, and potentially the role of the DLPFC in both, it was not possible to disentangle the effect of tDCS on each of these using this task. Having said that, the task demands in terms of WM were minimal, considering that the memory representation did not have to be updated throughout the session.

6.2 Inter-individual variability in tDCS response

As expected, inter-individual variation in response to tDCS was observed (Wiethoff et al., 2014). Most patients in the sample showed a positive response to the administration of tDCS, which was evident in a reduction of missed targets. For some individuals, a response to tDCS could not be demonstrated, as their task performance was identical in real and sham stimulation condition. A minority of patients appeared to show detrimental effects and got worse with brain stimulation. It should be stated, however, that some of this apparent improvement/worsening may be random noise.

The individual factors that predispose healthy individuals to a positive response are not fully understood. It has been proposed that this may at least partially relate to cortical morphology (Filmer, Ehrhardt, Shaw, Mattingley, & Dux, 2019). If tDCS were to become a potential treatment in spatial neglect, research efforts should be made to establish what is the dose of current that reaches the cortex in the healthy and damaged brain, and tailor treatment to the individual. This would also be crucial to ensure that some individuals are not missing the opportunity to benefit from a potential treatment, for instance, because they have not received a

sufficient dose, or because they have not received it for an adequate time. In the patient population that took part in the present study, it could be possible that a brain injury damaging a particular brain region or network may have prevented patients to benefit from the treatment. This issue will be systematically addressed in Chapter VI, where the neural correlates of tDCS responsiveness, both anatomical and functional, will be examined.

6.3 tDCS effect on lateralised attention deficits of neglect

The effect of tDCS described above did not show evidence of any transfer to other tasks indexing lateralised measures of attention. No effect of stimulation on the cancellation tasks or the line bisection task emerged. That is, offline tDCS delivered to the right prefrontal cortex did not seem to improve performance on clinical measures of the spatial bias.

It should be noted that there are some factors that may have potentially limited my ability to establish an effect of tDCS on these standard neglect tests, and to determine whether an improvement in performance on the vigilant attention task might directly relate to improvement on standard neglect tasks. First, testing for lateralised deficits was performed *offline*, which makes it less likely to be affected by the application of brain stimulation as compared to the vigilant attention task which was performed *online*.
In addition, and related to the previous point, any improvement on clinical tests would have had to be demonstrated as a difference between pre- and post-tDCS across two sessions, rather than a straightforward real vs. sham tDCS comparison. Finally, another limitation of this approach could be that most patients were close to ceiling on some of these tasks already before any stimulation was administered, leaving little room for improvement. For instance, our cohort of chronic right-hemisphere stroke patients performed the star cancellation task at an average accuracy level of 88% level in the pre-evaluations.

6.4 Limitations and future directions

The majority of stroke patients assessed on stroke wards were excluded from participating in the study because they did not meet inclusion/exclusion criteria that were in line with current recommendations for tDCS in this population. This issue can strongly limit the number of individuals who could potentially take part in a clinical trial, whereby multiple sessions of tDCS are offered (for a discussion on the issue of feasibility of tDCS as a remediation for post-stroke deficits, see Learmonth et al., 2020). More studies are needed to ensure that tDCS recommendations are up to date with current evidence, and to investigate the effects of tDCS in cases of seizures and/or surgically restored skull, which are relatively common in this clinical population, but currently represent exclusion criteria for brain stimulation studies as a precaution.

As discussed before, some variability in the response to tDCS emerged. Further studies examining the effects of increasing the tDCS dose would be useful in characterising tDCS response. In the HD-tDCS literature, studies vary for example in respect to the intensity and duration of stimulation, which was kept relatively low (1mA) and short (10minutes) in the present study, but that could possibly be safely increased in future investigations, and potentially affect performance in individuals that did not respond at smaller doses.

A phenomenon related to vigilant attention is that of mind wandering, which is often defined as an internally triggered phenomenon whereby individuals zoom-out and sustain task-unrelated thoughts (Smallwood & Schooler, 2006). The task used here, however, is not best suited to study momentary lapses of attention, because those might not be noticed if they were to occur when non-targets (which do not require any response from participants) were presented. RT variability was used in this study as a broad indicator of the presence of lapses of attention. However, as noted above, temporal precision at which fluctuations can be detected with this task is low. Other tasks such as the so-called BLAST paradigm, by allowing tracking at second-to-second temporal precision, might be more suitable

for this approach, and would allow exploration of whether the montage used here also affects mind wandering (Petton et al., 2019).

An absence of transfer of the beneficial effect of stimulation on measures of the spatial bias was observed. In addition to the points discussed above in Section 6.3, it should be noted that the spatial bias was not a primary outcome of the study. Consequently, the study was not powered to detect a significant change in standard measures of neglect; instead, it was fully powered to detect a change in non-lateralised measures of attention following stroke. Further work with adequate numbers is required to fully evaluate whether there is a therapeutic role for prefrontal tDCS in the modulation of lateralised attentional deficits following stroke. Experimentally, one could directly explore this issue by examining the online effect of prefrontal tDCS on a continuous task which has a spatially lateralised component.

7 Conclusions

In a randomised, double-blind, sham-controlled, crossover study, the effects of a single dose of tDCS targeted to the right dorsolateral prefrontal cortex were examined. Twenty-two stroke patients with right hemisphere brain damage were tested on an attentional task during brain stimulation, and on standardised clinical measures of neglect performed before and after the application of tDCS.

The study provided support for a role of prefrontal tDCS in modulating attention in this clinical population. A significant reduction in the total number of missed targets on the attentional paradigm was observed for the tDCS versus the placebo condition. Furthermore, the study suggested that offline tDCS applied to the right dorsolateral prefrontal cortex did not significantly affect performance on standardised clinical measures of neglect. The benefit of a single application of tDCS was observed in a sensitive and specific computerised task.

The clinical value of the targeted application of tDCS in neglect rehabilitation could not be fully assessed with this design, and additional work is necessary to determine whether the repetitive application of tDCS over consecutive sessions may be efficacious in modulating activities of daily living long-term in this clinical group. However, results from this proof-of-principle study are encouraging, and the methodology used was proved feasible and safe to use with stroke patients.

8 References

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Chapter VI:
Anatomical and functional correlates of tDCS responsiveness

1 Introduction

In the previous two chapters, the targeted application of tDCS over the right dorsolateral prefrontal cortex was found to improve target detection in a sample of healthy younger and older adults (Chapter IV) and stroke patients affected by attentional difficulties (Chapter V). The aim of the work described in this chapter was to examine the neural correlates of vigilant performance and tDCS responsiveness in these populations.

Two approaches were employed. In Study 1, voxel-based lesion symptom mapping (VLSM) was used to identify the brain regions that were most likely to be associated with task performance and response to tDCS in stroke patients. In Study 2, a new experiment was carried out to examine the functional connectivity correlates of tDCS responsiveness, across the life span and in right-hemispheric stroke.

2 Study 1: Lesion Anatomy

A lesion anatomy study was carried out to relate patient behaviour to the brain regions damaged by stroke. Lesion maps were delineated for the 22 patients with right-hemispheric stroke who took part in the crossover experiment described in Chapter V. For 18 of these patients, detailed anatomical maps were available as part of their participation in the imaging study described below in Section 4 (Study

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Preliminary findings from the imaging study described in this chapter have been published in abstract form. Olgiati, E., Violante, I.R., Li, L.M., Faraj, A., Sinclair, T., Crow, J., Wise, R., & Malhotra, P. (2019). Targeted brain stimulation to ameliorate vigilance in stroke: a combined tDCS-fMRI approach. *Brain Stimulation* 12(2):573.

2: Functional Connectivity). For 4 patients who did not take part in the imaging study, clinical imaging was used for lesion mapping.
The methodology used for lesion delineation and spatial normalisation has been described in detail in Chapter II, Section 3.7. Briefly, lesions were traced on native space and images were normalised to a common template, the MNI space, allowing statistical interrogation of lesion data across the group. The final product, spatially normalised three-dimensional binarized arrays, was a set of lesion maps reflecting the damaged locations for each patient.

2.1 Analysis

The software package MRIcron was used to draw a lesion overlap map for all patients and extract lesion volume for each patient (Figure 6.1) (https://www.nitrc.org/projects/mricron; Rorden, Karnath, & Bonilha, 2007). Volume was then correlated using Pearson's correlation coefficient with behavioural performance to establish the role of lesion size in the behaviours of interest, namely vigilance task performance and response to tDCS.

The importance of lesion location was then examined by using the following two voxelwise lesion analysis techniques.

2.1.1 Univariate statistical comparison

A univariate voxelwise statistical comparisons method was used to examine how lesions in different voxels influenced patients' behavioural scores on the vigilance task. This analysis was aimed at determining what brain regions were associated with reduced ability to sustain attention to spatial locations, and also what brain regions were related to lack of response to the application of targeted prefrontal tDCS. Lesion maps for the 22 patients were therefore correlated with two sets of behavioural data: mean number of omissions across the two sessions (predictor 1) and the difference between real and sham (predictor 2). For each predictor, task performance for the group of patients in whom a given voxel was damaged was statistically compared to the data of patients in whom that same voxel was intact, generating a voxel-wise map of statistical significance.

To perform this analysis, a General Linear Model was set up using the NiiStat toolbox (https://www.nitrc.org/projects/niistat/), which required MATLAB (Mathworks, Release 2012b) and SPM (*Statistical Parametric Mapping*, 2007). To ensure sufficient minimum lesion overlap, voxels damaged in less than 10% of the patient group (2 patients) were excluded (Karnath, Sperber, Wiesen, & de Haan, 2019). To correct for multiple comparisons, result maps were corrected using permutation-based thresholding (Mirman et al., 2018). Permutation thresholding, used to control for the family-wise error rate, was set to 5000 (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). All statistical tests performed were one-tailed, under the assumption that brain injury would not have led to improved test performance.

The toolbox NiiStat allowed a region-of-interest (ROI)-based approach to analysis: the AALCAT atlas was used, which is a union of the AAL atlas, including 116 grey-matter ROIs (Tzourio-Mazoyer et al., 2002), and the CAT atlas, comprising 34 white-matter ROIs (Catani & Thiebaut de Schotten, 2008). Accordingly, data were segmented into 150 brain regions and the mean percentage of damage per each region was calculated. The behavioural data were then adjusted for the variability described by the overall lesion volume: total lesion volume was computed for each individual, regressed with each behavioural variable, and the subsequent analysis was based on the residual variability of the behaviour.

2.1.2 Lesion subtraction analysis

Lesion subtraction analyses were performed with MRIcron, dichotomising the continuous-level behavioural measurement into 'responders', i.e., patients who showed benefit from the application of tDCS, and 'non-responders', i.e., patients who showed no response to the application of tDCS, or had their performance worsen when tDCS was applied (e.g., see Lee et al., 2019). The lesion overlap map of responders was subtracted from the lesion overlap map of non-responders. For each voxel, the percentage of responders who had a lesion at the voxel was subtracted from the percentage of non-responders that had a lesion at the voxel.

The resulting map showed, for each voxel, the percentage relative frequency difference between the two groups, allowing to distinguish between regions that were often damaged in patients and regions that were specifically associated with the behaviour of interest (Rorden & Karnath, 2004).

2.2 Results

A colour-coded lesion overlap map of injured voxels across all patients was created to provide an overview of all lesioned brain areas in the sample of 22 patients (Figure 6.1).

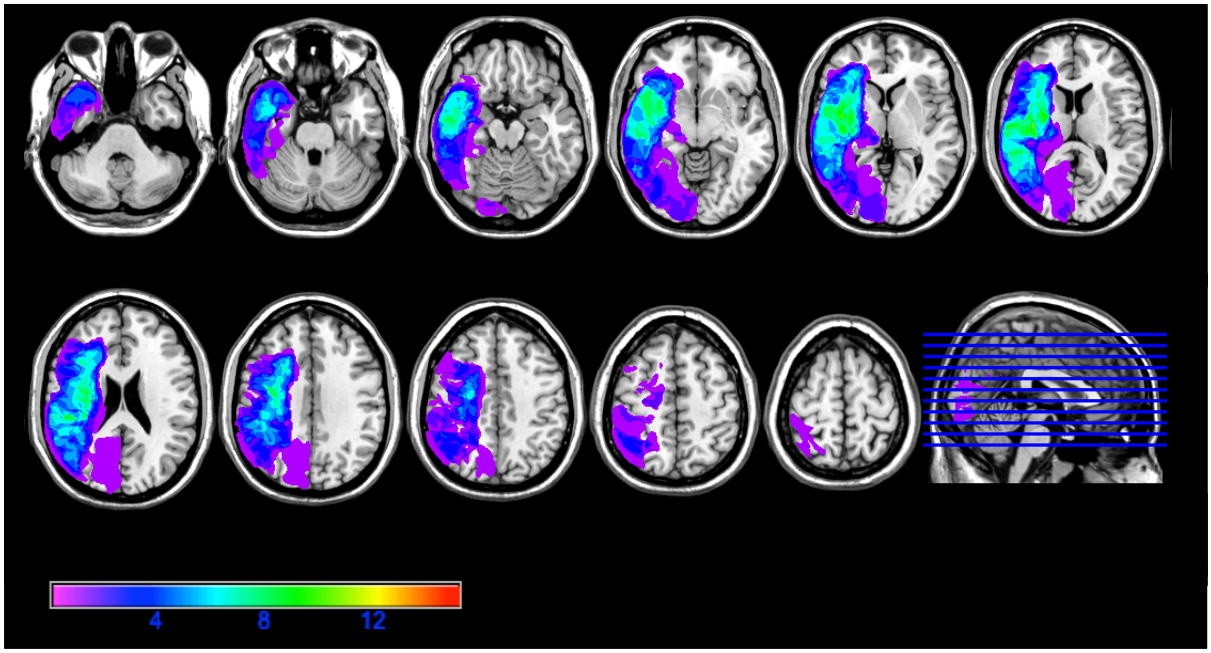


Figure 6.1: Lesion overlay plot for 22 patients with right-hemisphere stroke
Overlay map of individual stroke lesions. Colour scale shows the number of patients having a lesion in each voxel. The majority of patients had a stroke in the territory of the right middle cerebral artery. The map shows the wide lesion distribution across the right hemisphere of the brain, including all four cortical lobes and subcortical regions.

Lesion volume for each patient was extracted (Table 6.1) and correlated with behavioural variables. No significant correlation was found between lesion volume and average number of omissions made on the vigilance task [r(22)=2.11, p=.346]. Likewise, no significant correlation emerged between lesion volume and response to tDCS, as measured by difference in the total number of omissions between real

and sham stimulation [r(22)=.312, p=.157]. This lack of relationship suggests that lesion location, more than lesion size, is likely to be a crucial determinant of performance and also of response to tDCS.

Results of voxelwise lesion-symptom mapping analyses, revealing the statistical contribution of lesion location to vigilant performance and tDCS responsiveness, are described in the two sections below.

2.2.1 Neural correlates of task performance

To obtain insights into the neural correlates of vigilance task performance, a group comparison between patients having a lesion in each ROI (from the AALCAT atlas) and patients having no lesion in that area was performed, using the average number of omissions (computed as mean omissions between real and sham tDCS) as input for the model. Two regions survived thresholding ($p<.05$), indicating that lesions in the right cingulum (a C shaped collection of fibers connecting regions of the frontal lobe with the parietal and medial temporal lobes) and the corpus callosum (the largest commissure connecting the two hemispheres) were positively associated with the number of task omissions (respectively, $z=3.30$ and $z=3.44$) (Figure 6.2). When lesion volume was regressed out, the same regions emerged, confirming that in these two areas, higher lesion load led to worse behavioural performance. Damage in other areas was not associated with behavioural impairment.

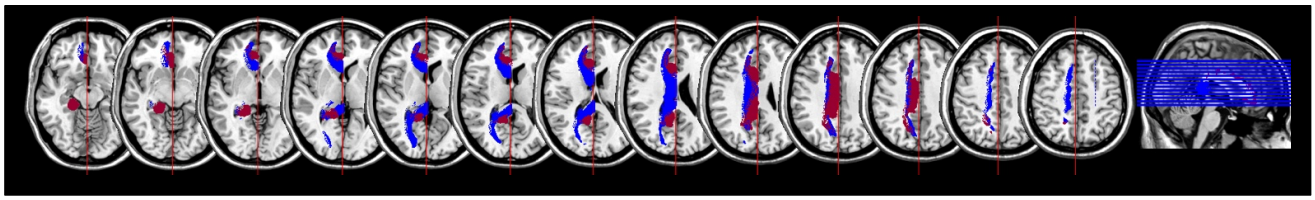


Figure 6.2: Statistical topography resulting from VLSM analysis
The analysis included 22 patients with right hemisphere damage, relating lesion location with performance on a behavioural task. Shown are statistically significant areas in axial view: damage to the right cingulum (burgundy) and the corpus callosum (blue) were associated with poorer performance on the vigilance task.

In summary, this analysis revealed that, in the tested population of 22 individuals with stroke, there was a significant correlation between the scores on the vigilance task and the amount of white matter damage across long fibre bundles that run in an anteroposterior direction or that connect the two hemispheres.

2.2.2 Neural correlates of tDCS responsiveness

A second aim was to determine the anatomical correlates of tDCS responsiveness, i.e., identifying what brain regions were associated with a reduced behavioural response to brain stimulation. Univariate analysis, which was conducted by feeding into the model the difference in omissions during sham vs real tDCS and the lesion maps for individual patients, did not identify any specific brain regions associated with response to tDCS.

Patients were then split into two groups, responders (n=13) and non-responders (n=9) to tDCS, with the latter group including cases where a response could not be demonstrated (see Chapter V, Section 4.1.3) (Figure 6.3) (Table 6.1).
Mean lesion volume was not significantly different between the group of responders and non-responders (respectively, 65.68cm^3 and 45.35cm^3), as tested via a one-way ANOVA [F(1,21)=.797, p=.383].

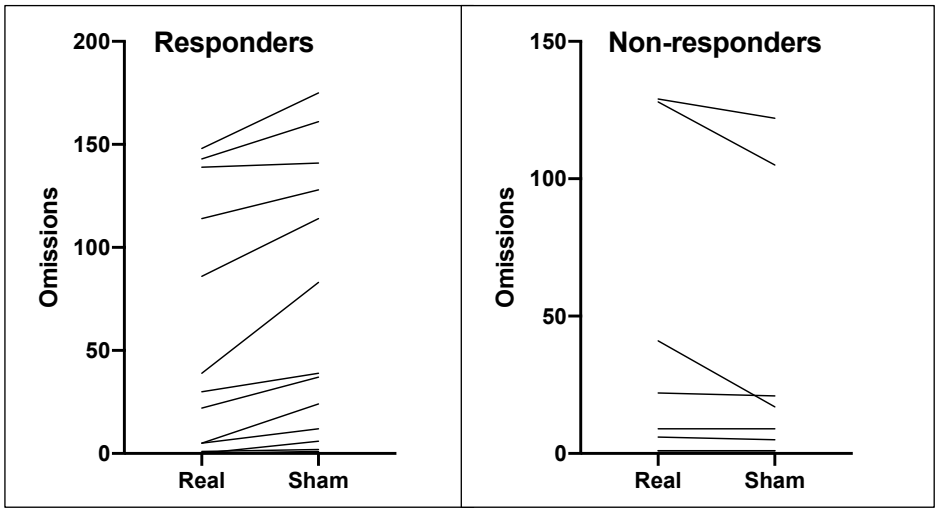


Figure 6.3: Task omissions for responders (n=13) and non-responders (n=9)
Individual patient performance on the vigilance task during real and sham tDCS.

Patients	Omissions real	Omissions sham	Omissions mean	tDCS Benefit	Lesion volume
R1	39	83	61	44	167.46
R2	143	161	152	18	21.40
R3	22	37	29.5	15	151.73
R4	139	141	140	2	106.88
R5	0	1	0.5	1	1.66
R6	1	2	1.5	1	1.11
R7	5	12	8.5	7	100.01
R8	86	114	100	28	18.98
R9	114	128	121	14	73.60
R10	5	24	14.5	19	14.48
R11	30	39	34.5	9	62.59
R12	0	6	3	6	22.29
R13	148	175	161.5	27	111.63
mean	*56.31*	*71*	*63.65*	*14.69*	*65.68*
NR1	1	1	1	0	16.91
NR2	6	5	5.5	-1	27.85
NR3	1	1	1	0	1.74
NR4	129	122	125.5	-7	34.25
NR5	0	0	0	0	140.70
NR6	128	105	116.5	-23	55.07
NR7	41	17	29	-24	78.99
NR8	22	21	21.5	-1	1.24
NR9	9	9	9	0	51.39
mean	*37.44*	*31.22*	*34.44*	*-6.22*	*45.35*

Table 6.1: Task performance and lesion volume in cubic centimetres (cc) for patients in the group of tDCS responders (R) and non-responders (NR)

A lesion subtraction analysis was then carried out with the aim of directly comparing lesion profile between the group of responders and non-responders. Figures 6.3 A and B show lesion overlap for each group. Strokes involving the MCA occurred in both groups; the only two patients with a PCA stroke were part of the non-responders group (NR4 and NR6). In panel C, regions of mutual involvement in responders and non-responders are shown. Panel D shows the subtraction plot, identifying regions more commonly damaged in non-responders.

These were the right thalamus and the right postcentral gyrus, circled in yellow in Figure 6.4 D (MNI coordinates: X=20, Y=-20, Z=12; X=55, Y=-22, Z=28). Lesioned voxels here were associated with lack of response to the administration of tDCS.

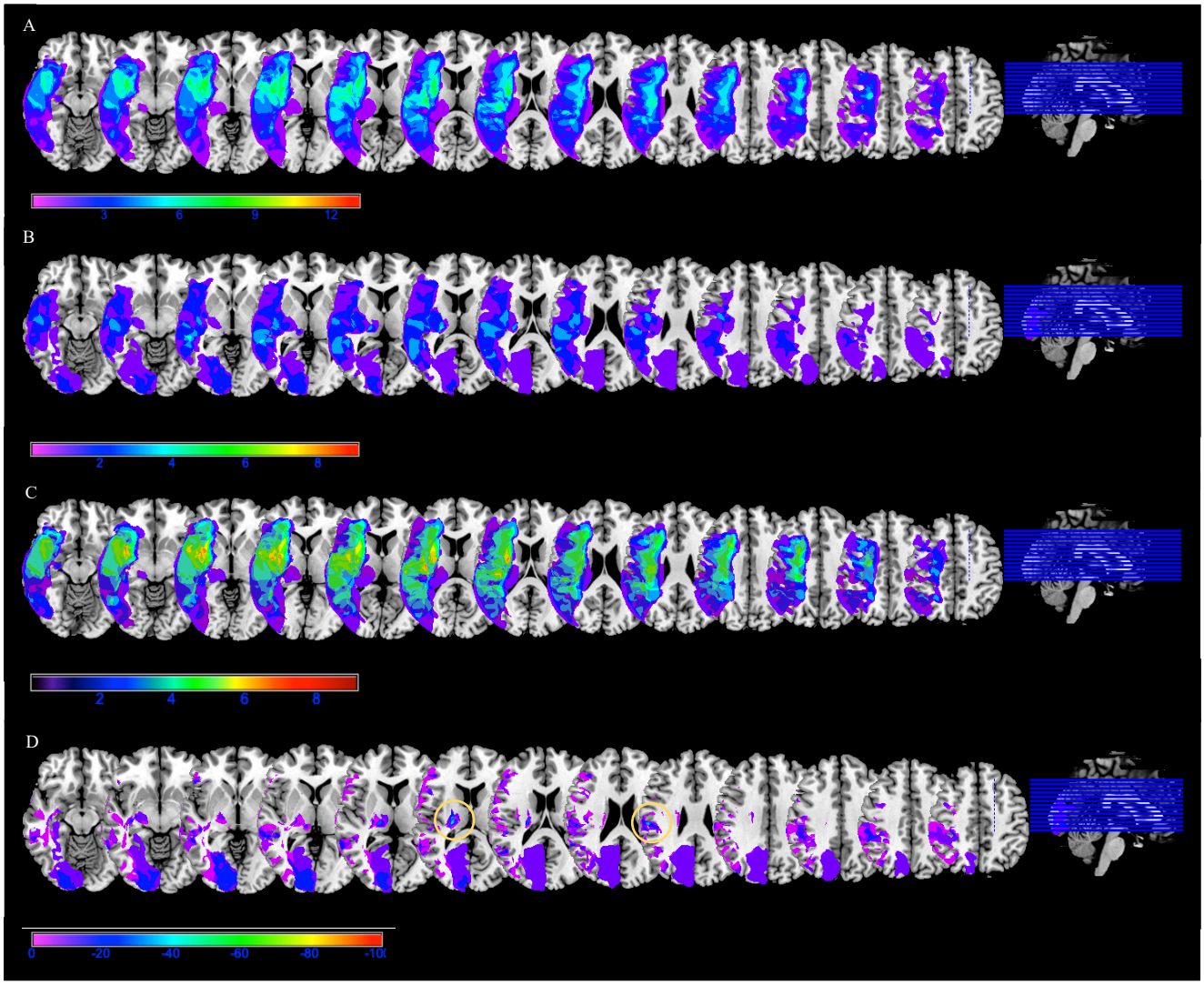


Figure 6.4: Lesion overlap and subtraction analysis for patients
A. Lesion overlap for patients with a right-hemispheric stroke who benefitted from the application of targeted tDCS (responders). B. Lesion overlap for patients with a right-hemispheric stroke who did not demonstrate benefit from the application of targeted tDCS (non-responders). C. Intersection map showing regions of mutual involvement. D. Lesion subtraction map showing the percentage of lesion overlap of non-responders after the subtraction of the overlap of responders. In light blue (circled in yellow), areas where a lesion was associated with a lack of benefit from tDCS. Colour bars reflect the relative frequency of damage.

In summary, the lesion subtraction analysis showed some indication that the right thalamus and the right postcentral gyrus may be key regions in determining the effect of targeted prefrontal tDCS. Patients with lesions involving these regions were less likely to benefit from the intervention.

3 Ad interim discussion

3.1 Lesion anatomy of vigilant attention to spatial locations

Lesion mapping techniques with structural brain imaging were employed to investigate whether a relationship was present between lesion volume, lesion location and task performance. While lesion volume was not associated with task performance, damage to specific locations was associated with reduced ability to sustain attention to spatial locations. A role for two large white matter bundles, the right corpus callosum and the right cingulum, emerged, suggesting that the underlying anatomy of vigilant attention performance may be linked to disconnections between hemispheres or between right frontal and parietal areas.

A potential role of the corpus callosum in vigilant behaviour had already been put forward. Dimond studied six individuals who had complete resection of the corpus callosum, as well as of the hippocampal and anterior commissures (Dimond, 1979). On a 30-min visual vigilance task, patients demonstrated a clear vigilance decrement, with a superiority of the right hemisphere for this task. A role for the corpus callosum in maintaining attention is also supported by studies in healthy individuals. Rueckert and Levy for instance found a measure of efficiency of interhemispheric transfer to positively correlate with performance over a 10-minute task (Rueckert & Levy, 1996). In addition, researchers found that children with low callosal efficiency showed a vigilance decrement in RT and target detection in the second half of a task, as compared to another group of children with higher callosal efficiency on the task.

In addition, the present findings extend those by Malhotra and colleagues, who provided initial evidence for a role of the white matter laying deep to the

temporoparietal junction in sustaining attention to spatial locations over time in stroke patients (Malhotra, Coulthard, & Husain, 2009). A potential role of the cingulum in vigilance arrives from research conducted in other disease groups. By imaging patients with traumatic brain injury, researchers suggested a link between sustained attention impairments and an increase in DMN activation, particularly within the precuneus and the posterior cingulate cortex, strongly connected to the prefrontal lobe via the cingulum (Bonnelle et al., 2011). Nestor and colleagues studied 30 patients affected by schizophrenia and 30 age-matched controls (Nestor et al., 2007). Patients, as compared to controls, demonstrated decreased alertness, as measured by the Attention Network Test (ANT). In addition, there was a suggestion that smaller right cingulum bundle volume, measured with DTI, correlated with reduced alertness – this relation however did not hold when covarying for medication and illness duration.

The analysis discussed above captured the importance of lesion location, rather than extent of a lesion, for vigilance - volume did not correlate with task performance, and when regressed out of the model the importance of location over volume was confirmed. It is worth emphasising that the anatomy of the stroke population studied here was largely subcortical. This is in line with recent findings in the literature, confirming that stroke topography is predominantly subcortical (Corbetta et al., 2015). A tractography study could potentially reveal whether vigilance deficits are associated with disconnection within specific frontoparietal tracts. DTI sequences were collected as part of the imaging study described in section of the current chapter; however, their evaluation is beyond the scope of this thesis. Further research focusing on tract disconnections could for instance examine whether specific white matter tracts (e.g., the superior longitudinal fasciculus) are particularly likely to be disrupted in patients with vigilance impairment.

3.2 Lesion anatomy of tDCS responsiveness

A second scientific question aimed to explore whether changes in vigilance during brain stimulation were related to neuroanatomy. To this aim, lesion anatomy methodology was employed to investigate the precise lesion profile in those patients who showed a lack of response to a potential treatment involving the administration of tDCS. Answering this question might allow the characterisation of treatment responders versus non-responders, and potentially advance our understanding of the interindividual differences in response to tDCS in a clinical population affected by brain damage.

When tDCS responders and non-responders were contrasted in a lesion subtraction analysis, it was possible to identify two regions that, when damaged, precluded patients to benefit from the application of tDCS. These were the right thalamus and the right postcentral gyrus. Their potential role in mediating the response to tDCS will be discussed in Chapter VII, Section 3.6.

A further line of investigation relates to the areas anatomically connected to the stimulation site. Although it was ensured that all participants had intact dorsolateral prefrontal cortex, which was indeed targeted by tDCS, it is still possible that the level of brain damage was such that this region's connectivity was disrupted by white matter loss, making it functionally isolated from other intact regions. It would be interesting for future investigations into tDCS responsiveness to take white matter tract volume into account.

3.3 Study limitations

To perform the lesion anatomy analysis, an index of vigilant performance was arrived at by averaging the number of omissions made on the task across the two different sessions (real and sham tDCS). This decision was taken on the grounds that treatment order was not found to be a significant determinant in performance, and was not found to interact with stimulation in patients (Chapter V, Section 4.1.2.4). Another possibility would have been to take solely performance on the first day of testing, but this would be problematic as patients received different

treatments in session one – some performed the task whilst receiving real tDCS, others whilst received sham tDCS. Ideally, a study design with a formal baseline session – with participants performing the task without receiving any stimulation - would have been optimal to investigate this relationship. It should be noted, however, that the primary aim of the current thesis was not to determine the anatomical locus of response to tDCS, but to examine its potential to augment vigilant attention across the lifespan and in right-hemispheric stroke.

Another study limitation is related to the split into responders and non-responders, which is always somewhat arbitrary. A similar approach, which allows differentiation of the effect of lesion anatomy on response, has been used by previous studies (Li et al., 2018; Malhotra, Parton, Greenwood, & Husain, 2006). In this study, any increase in targets detected during real stimulation was considered a response to the application of tDCS. It nevertheless remains to establish by future research what can be considered a minimal clinically important difference. With a larger group, it would have been possible to carry out further analysis to assess the role of different regions in blunting response to tDCS. It in indeed recommended that a sample size ranging between 30 and 100 patients is used to perform voxelwise statistical comparisons (Karnath et al., 2019).

Another issue for consideration relates to an underlying assumption of the univariate approach to lesion-mapping analysis used here. This approach assumes that every voxel is independent of one another, increasing the potential to find false positives because a large number of comparisons are carried out. The most robust method for correcting for the family-wise-error, namely permutation thresholding, was used to account for this issue (Mirman et al., 2018).

Having explored anatomical correlates of vigilant performance and tDCS responsiveness, brain network response to the targeted application of prefrontal tDCS application will be examined in the next section.

4 Study 2: Functional Connectivity

Recent methodological advances allow for the delivery of tDCS in an MRI environment (for resting-state fMRI principles and methodology, see Chapter III, Section 4). A separate multimodal imaging study was carried out to examine directly whether tDCS induced any modulations in intrinsic brain activity; that is, activity in the absence of any explicit input/task. This study was conducted in a sample of healthy individuals across the lifespan and also in patients with right-hemispheric stroke. This allowed investigating how Resting State Networks (RSN), comprising spatially separated but functionally linked anatomical regions that are continuously communicating, may be affected by the targeted application of brain stimulation over the right dorsolateral prefrontal cortex. For the patient group, changes in functional connectivity during tDCS in this experiment were then examined in the context of behavioural response to tDCS in the crossover study presented in Chapter V.

4.1 Methods

4.1.1 Sample size

Traditional power analyses were not used to determine group sizes, because of the difficulty in specifying the expected variance for fMRI studies. Nevertheless, previous studies have shown that reliable and sensible results can be obtained in patient populations with 10-15 subjects when studying motor functions (Stagg et al. 2012; O'Shea et al. 2014; Fregni et al. 2005). Considering that the variability of responses is likely to be higher with cognitive tasks, and that cognitive studies using brain stimulation in both healthy and patient populations tend to recruit at least 20 individuals per group (P S Boggio et al. 2007; Weltman & Lavidor 2013; Marshall et al. 2004; Paulo S. Boggio et al. 2007; Dockery et al. 2009; Fecteau et al. 2007), it was decided to recruit a minimum of 20 participants per group and to acquire two fMRI runs (one real and one sham tDCS) per participant during a single session in the scanner, to allow within-participant (versus between-groups) comparisons.

4.1.2 Participants

Healthy younger adults (n=20), healthy older adults (n=23) and chronic stroke patients (n=23) participated in the imaging study. tDCS-specific and patient-specific inclusion and exclusion criteria were applied (see Chapter IV, Section 3.1 and Chapter V, Section 3.2), in addition to specific contraindications to MRI scanning such as claustrophobia (Appendix II). Following data acquisition, one older adult and one stroke patient were excluded from any analysis due to incidental findings (i.e., a frontal meningioma and a large cyst). Sample characteristics, including demographic and clinical data for patients, are presented in Tables 6.2 and 6.3. The final sample included 20 younger adults (mean age=28.3±6.3 years old, age range 20-39 years old, F=12), 22 healthy older adults (mean age=63.00±8.14 years old, age range 49-80 years old, F=10) and 22 stroke patients (mean age=65.77±12.37 years old, range 45-85 years old, F=11).

The study was approved by the Central London Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before participation.

YOUNGER ADULTS

ID	Age	Sex	Handedness
Y1	32	F	R
Y2	33	F	R
Y3	25	F	R
Y4	39	M	R
Y5	39	M	R
Y6	33	F	R
Y7	29	F	R
Y8	23	F	R
Y9	28	F	R
Y10	30	F	R
Y11	27	F	R
Y12	37	M	R
Y13	28	F	R
Y14	24	F	R
Y15	21	M	R
Y16	35	M	R
Y17	20	M	R
Y18	22	F	R
Y19	20	M	R
Y20	21	M	R

OLDER ADULTS

ID	Age	Sex	Handedness
O1	60	M	R
O2	73	M	R
O3	64	M	R
O4	55	F	R
O5	52	F	R
O6	63	M	R
O7	80	F	R
O8	77	F	R
O9	49	F	R
O10	67	F	R
O11	49	F	R
O12	64	M	L
O13	64	M	R
O14	53	M	R
O15	63	M	R
O16	62	M	R
O17	60	F	R
O18	67	M	R
O19	71	F	R
O20	63	M	R
O21	64	M	R
O22	66	F	R/L

Table 6.2. Demographics for the younger and older groups of healthy participants who took part in the tDCS-fMRI study
Sex: F=female, M=male. Handedness: R=right L=left.
In grey background, participants who did NOT take part in the crossover experiment described in Chapter IV. The remaining 13 younger and 8 older adults took part in both studies.

STROKE PATIENTS

ID	Age	Sex	Handedness	Aetiology	Lesion location	Months post-stroke	M	SS left	SS bilateral	V left	V bilateral	Stars
P1	77	F	R	I	P-O	84	1/3	10/10	10/10	10/10	10/10	NA
P2	77	M	R	I	F-T-P	48	1/3	10 /10	0/10	10/10	10/10	24
P3	77	F	R	I	F-T-P, BG	10	3/3	6/10	3/10	10/10	0/10	0
P4	71	M	R	I	IC	27	0/3	10/10	7/10	10/10	10/10	98
P5	69	F	L	H	Th, BG	30	1/3	10/10	10/10	10/10	10/10	100
P6	57	F	R	I	T-O, h, Th, CC	6	1/3	10 /10	10/10	10/10	9/10	28
P7	54	F	R	I	CR, LN	5	2/3	10 /10	10/10	10/10	10/10	100
P8	63	M	R	I	T-P, insula, BG, fO	5	1/3	10/10	0/10	10/10	0/10	80
P9	85	F	R	I	BG, insula, fO	36	3/3	9/10	10/10	10/10	7/10	73
P10	75	F	R	I	F-T, IC, LN	26	1/3	10/10	3/10	5/10	5/10	16
P11	80	F	R	I	BG, IC	3	0/3	10/10	10/10	10/10	10/10	55
P12	46	F	L	I	F-T-P	48	1/3	8/10	0/10	10/10	5/10	62
P13	45	M	R	H	BG, CR	23	3/3	0/10	0/10	10/10	10/10	42
P14	70	F	R	I	F-T-P, insula, fO, tO	3	0/3	10/10	10/10	10/10	5/10	NA
P15	76	F	R	I	F-T-P, CS	3	1/3	10/10	0/10	9/10	0/10	11
P16	54	M	R	H	T	5	0/3	10/10	10/10	10/10	8/10	98
P17	53	M	L	I	F-P, insula	3	0/3	10/10	0/10	10/10	10/10	0
P18	85	M	R	I	P-O	3	3/3	10/10	10/10	0/10	0/10	22
P19	65	M	R	H	Th, IC	19	1/3	10/10	10/10	10/10	10/10	2
P20	59	M	R	I	T-P, insula	3	3/3	10/10	0/10	10/10	10/10	37
P21	55	M	R	H	Th	6	0/3	10 /10	10/10	10/10	10/10	24
P22	54	M	R	I	F, CR, insula, fO	3	1/3	10/10	10/10	10/10	10/10	79

Table 6.3: Demographics for the group of stroke patients who took part in the tDCS-fMRI study

Sex: F=female, M=male. Handedness: R=right L=left.

In grey background, patients who did NOT take part in the crossover experiment described in Chapter V. The remaining 18 patients took part in both studies. Stars: proportion of identified targets on the star cancellation task performed on the ward in acute/post-acute stage of illness. NA=not available.

4.1.3 Study design

4.1.3.1 Pre-scanner

Before entering the scanner, a brief behavioural testing session was carried out. This included the vigilance task (Chapter IV, Section 3.3.1) and, for patients only, three clinical screening measures for the presence of spatial neglect, namely the star cancellation, Mesulam cancellation and line bisection task (Chapter II, Section 2.2.2). These measurements were not analysed as part of the present thesis, which is focussed on vigilant attention and its susceptibility to the application of targeted tDCS. Participants were then familiarised to the stimulation procedure in a habituation phase, when stimulation was delivered for 10s at half intensity (0.5mA) followed by 10s at full intensity (1mA).

4.1.3.2 MRI acquisition

The imaging protocol included two resting-state functional MRI runs, during which real and sham tDCS were delivered intrascanner. Brain stimulation was set up for delivery in the scanner room as previously described in detail (Chapter III, Section 4.3). The two stimulation conditions, applied in counterbalanced order to minimize risk of any systematic bias, were separated by 10 minutes, during which participants underwent structural scans (see imaging protocol in Chapter II, Section 3.4). FMRI images were acquired while participants were at rest. Specifically, participants were instructed to keep their eyes closed and try not to fall asleep – this is due to the changes in BOLD amplitude observed when subjects fall asleep (Horovitz et al., 2008; Tagliazucchi & Laufs, 2014). Instead, they were asked to let their mind wander, trying not to think of anything in particular.

Immediately after each 10-minute run of tDCS-fMRI, participants were asked to state whether they thought they had received any stimulation or not, and to report the occurrence/ intensity of any adverse effects (i.e., itching, pain, metallic taste, burning, anxiety, anything else) on a scale from 1 to 5, where higher values describe more intense sensations (as in Li et al., 2019). This tool, adapted for intrascanner administration from the questionnaire developed by Brunoni and

colleagues (Brunoni et al., 2012), allowed the collection of critical information about tDCS blinding while participants were lying in the scanner, via the two-way intercom system (Appendix IX).

4.2 Functional connectivity analysis

Inter-regional correlations between signal fluctuations recorded 'at rest' reveal how components of large-scale distributed neural systems are coupled together. This is typically indicated with the term Functional Connectivity (FC), which describes the coupled and integrated performance of different brain regions (see review by Rogers, Morgan, Newton, & Gore, 2007).

In order to analyse functional images of 'resting' state networks, rigorous pe-processing was performed, which included conventional pre-processing (e.g., spatial smoothing) and noise-reduction steps (i.e., ICA-based clean-up). For a description of each pre-processing step performed on functional MRI data, see Chapter III, Section 4.4. Four-dimensional datasets were visually examined after every step of data pre-processing, ensuring no systematic error or artefact was present.

Before any statistical analysis, it was ensured that data for participants entering each model were not contaminated by large motion. As described in Chapter III, Section 4.4.3, FC quality-based exclusion criterion included having fewer than 255 usable frames. It follows that for each comparison, datasets of participants whose run did not obey this criterion were excluded from the analysis.

Independent Component Analysis (ICA) and dual-regression methodology were employed to study functional connectivity in 'resting' state fMRI (Filippini et al., 2009; Leech, Kamourieh, Beckmann, & Sharp, 2011; Zuo et al., 2010). This is a data-driven multivariate approach which allows the interrogation of fMRI data from an exploratory angle (Cole, Smith, & Beckmann, 2010). The pipeline summarising the pre-processing and analysis carried out on functional imaging data is displayed in Figure 6.5.

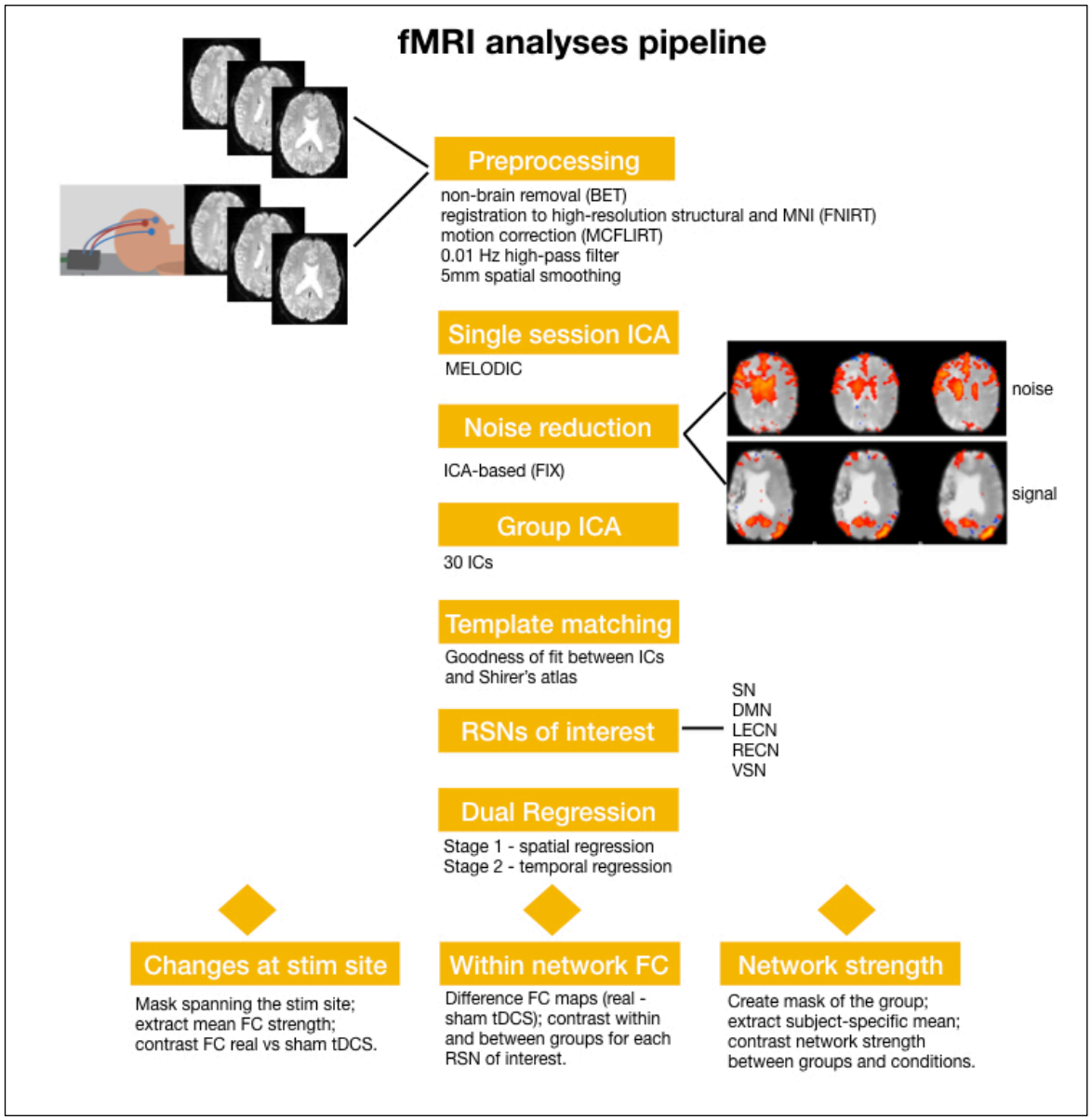


Figure 6.5: fMRI analysis pipeline

4.2.1 Group ICA

The first analysis step involved registering individual cleaned single-subject data (i.e., the output maps of single-subject ICA described in Chapter III, Section 4.4.4.1, which were in native space) to a common reference coordinate system, the standard MNI space. This was performed using the multi-stage non-linear registration tool (FNIRT) in FSL, with high-quality brain-extract structural T1 images for best results (Smith et al., 2004).

For each group, individual data were then temporally concatenated across subjects to create single 4D datasets using the MELODIC tool in FSL. MELODIC uses Independent Component analysis (ICA) to decompose the matrix of imaging data into a set of spatially and temporally independent features called components. Specifically, using a completely data-driven parcellation, voxels were separated into a set of nodes at a dimensionality of 30 components, and large-scale resting state networks (RSN) common across participants were identified.

The output from each group-ICA was always visually inspected for separating functionally interpretable, non-artefactual components from others noise components (e.g., head motion, CSF etc.). The FSL utility fslcc (i.e., fsl crosscorrelation) was used to spatially correlate each component's spatial map to a set of reference networks. The atlas that was used included 14 RSN, covering most of the brain cortical and subcortical grey matter and freely available online (Shirer, Ryali, Rykhlevskaia, Menon, & Greicius, 2011) (http://findlab.stanford.edu/functional_ROIs.html). Cross-correlation was preferred to manual selection of components because it is an objective technique that examines the fit of each data-driven identified network to reference networks derived from a robust connectivity-driven parcellation of cortex. Components that showed maximum overlap with the following 5 large-scale brain networks of interest were selected for further analysis:

- Salience network (SN)

This network primarily includes the anterior insula and the dorsal anterior cingulate cortex. It is a dynamic hub for detection and selection of salient stimuli (Menon, 2015).

- Default Mode Network (DMN)

This network is symmetrical and bilateral, involving the medial and lateral parietal, medial prefrontal and medial and lateral temporal cortices of the brain (Raichle, 2015). The DMN, also known as the task-negative network, shows increased activity when the individual is in a non-task state, and becomes less

active when the individual is engaged in a task; it appears to have a key role in processes such as introspection and mind wandering (Gusnard & Raichle, 2001).

- Left and Right Executive Control Networks (LECN and RECN)

These lateralised networks involve the left and the right dorsolateral prefrontal cortices (DLPFC) and the posterior parietal cortices. They are thought to be involved in goal-directed behaviour and cognitive control, whilst exhibiting anticorrelations during the resting condition (e.g., see Seeley et al., 2007).

- Visuospatial network (VSN)

This network comprises the posterior parietal cortex at the occipito-parietal junction, along the midline in the precuneus and posterior cingulate cortex and the frontal pole; it is engaged in orienting to salient visuo-spatial information (Beckmann, DeLuca, Devlin, & Smith, 2005).

These 5 RSN were considered to be the networks that, based on previous literature, had a spatial distribution that is compatible with the attention domain, or were networks that included the stimulated site (the right DLPFC) or its homologue. Functional connectivity analysis indeed focused on the components derived from the ICA that showed the higher cross-correlation with these five resting-state networks of interest.

Once group average ICA were obtained and the components for further analysis were selected, the following step was to relate group maps to the individual subjects. This was performed using the dual regression tool described in the next paragraph.

4.2.2 Dual regression

The dual regression approach was used to identify the subject-specific and condition-specific contributions to the group-level ICA (Filippini et al., 2009). This approach consisted of two stages. First, for each run separately, group-ICA spatial maps were regressed against each individual fMRI dataset to identify a set of

timecourses (stage 1). Subsequently, in stage 2, a temporal regression of the resulting timeseries against each subject's fMRI data was used to estimate run-specific spatial maps of functional connectivity.

Once a set of individual network maps had been obtained, comparisons were performed. I have performed both within and between-group analyses. For within-group analysis, performance was regressed against the average from their own group – as ageing and/or stroke may have systematic effects across networks. To compare FC between-groups, an average map was derived averaging across the three groups.

To quantify differences and carry out comparisons between conditions and groups, a first approach involved extracting mean connectivity strength within each network for each subject/condition by applying a mask on the output of dual regression (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Binnewijzend et al., 2012). Paired and unpaired t-tests were then used to compare mean connectivity strength between conditions/groups, as appropriate.

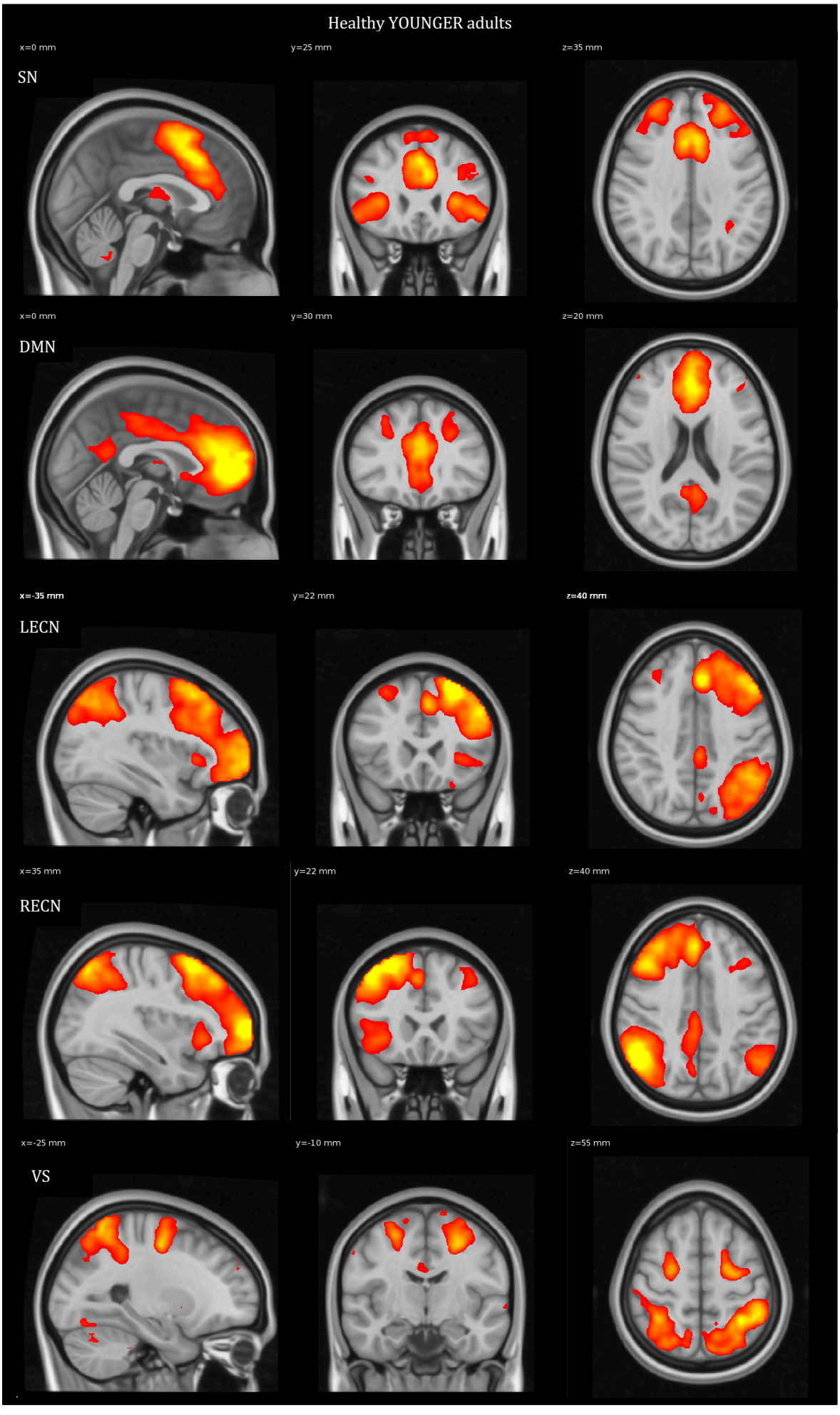
A second approach involved direct comparison of whole-brain FC spatial maps. This tested for shape and amplitude of low-frequency fluctuations, providing a voxel-wise measure of functional connectivity that reflects the correlation between activity of each voxel and the rest of the network (Beckmann et al., 2005). In order to do this, subject-specific spatial maps were compiled into a single four-dimensional file to perform non-parametric analysis.
Appropriate design models and contrast matrices were then created using the general linear model (GLM) framework. The dependent variable in the model contained all values from a single voxel across subjects' spatial maps, with statistical analyses repeated separately for each voxel. Comparisons (one-sample t-tests, paired and unpaired t-tests) were then run using FSL randomise nonparametric permutation testing (Winkler et al., 2014), with 5000 permutations and a threshold-free cluster enhancement (TFCE) method to control for multiple comparisons (Nichols & Holmes, 2002). The result was a group-level whole-brain

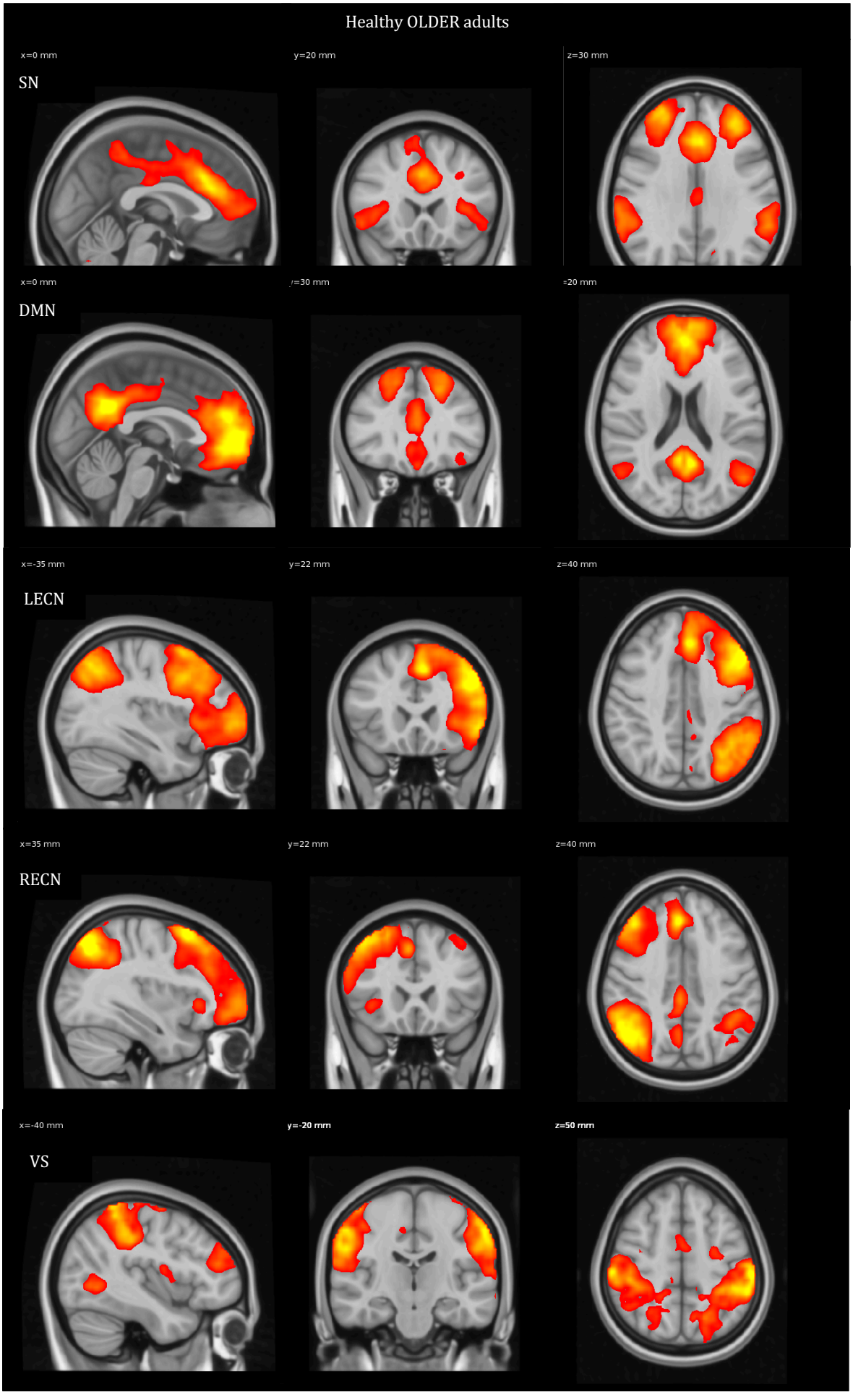
map, with significant clusters calculated using a p <.05 threshold. Corrected p-values obtained were fully corrected for multiple comparisons across voxels for each RSN in its own right - not across networks.

4.3 Results

A first set of Independent Component Analyses was performed separately on each group (younger adults, older adults and stroke patients) to ensure that the generated set of 30 resting state networks (RSN) could be reliably found across groups. A subset of patients (n=5) and healthy older adults (n=2) were excluded from this analysis because one or both runs (real or sham tDCS) was contaminated by excessive motion. ICA were therefore conducted in a total of 56 participants: healthy younger adults (n=20), healthy older adults (n=19), stroke patients (n=17).

Components were matched against a robust atlas (Shirer et al., 2011), and those which showed the higher cross-correlation value with the five networks of interests were visually inspected to confirm goodness of fit. The five resting-state networks of interest were indeed reliably and consistently found in the three groups of participants tested – for a rendering of each group mean RSN, generated using healthy and clinical populations, see Figure 6.6.


Healthy YOUNGER adults
x=0 mm
y=25 mm
z=35 mm
SN
x=0 mm
y=30 mm
z=20 mm
DMN
x=-35 mm
y=22 mm
z=40 mm
LECN
x=35 mm
y=22 mm
z=40 mm
RECN
x=-25 mm
y=-10 mm
z=55 mm
VS


Healthy OLDER adults
x=0 mm
y=20 mm
z=30 mm
SN
x=0 mm
DMN
x=-35 mm
y=22 mm
z=40 mm
LECN
x=35 mm
y=22 mm
z=40 mm
RECN
x=-40 mm
y=-20 mm
z=50 mm
VS

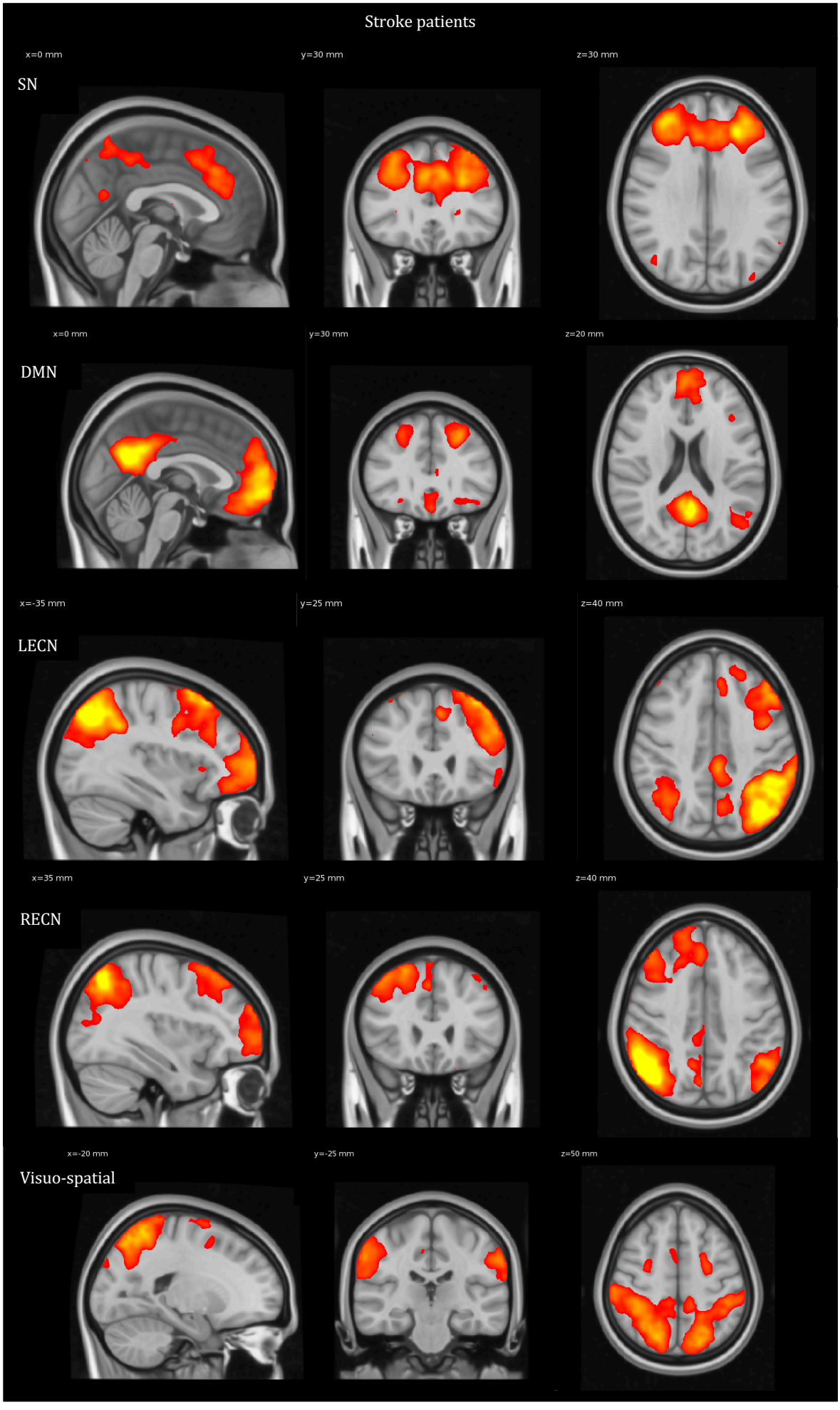

Stroke patients
x=0 mm
y=30 mm
z=30 mm
SN
x=0 mm
y=30 mm
z=20 mm
DMN
x=-35 mm
y=25 mm
z=40 mm
LECN
x=35 mm
y=25 mm
z=40 mm
RECN
x=-20 mm
y=-25 mm
z=50 mm
Visuo-spatial

Figure 6.6: Resting state networks of interest derived from resting state fMRI connectivity analysis using Independent Component Analysis (ICA) for healthy younger adults, healthy older adults and stroke patients
Regions within each network show similarity in their timecourse across the scan period.

4.3.1 tDCS-induced modulation in network activity

For each subject, two fMRI measures (10 minutes each) were acquired whilst participants were 'at rest' (i.e. not performing any task) - one during the administration of concurrent real tDCS, and one during sham tDCS. Analyses were performed with the aim of exploring if tDCS induced any modulation in functional connectivity, as compared to sham stimulation.

Dual regression steps (described in Section 4.2.2) were performed for each group of participants, and comparisons between conditions were performed as described in the next sections.

4.3.1.1 Local changes in connectivity during real and sham tDCS

A first interrogation related to whether the increased-focality tDCS montage utilised in this work induced local changes in functional connectivity (FC). To this aim, mean FC strength for the area underneath the electrodes was extracted and compared between real and sham tDCS.

This was implemented via the use of a mask centred around the stimulation target, i.e., the right dorsolateral prefrontal cortex, to restrict the analysis to this region. The anatomical mask corresponding to the tDCS electrodes was created using the software MANGO (http://ric.uthscsa.edu/mango/mango.html) (Multi-image Analysis GUI) by defining a 25 mm radius sphere that triangulated the EEG coordinates used to position each stimulation electrode (Figure 6.7). Coordinates for the EEG electrodes were obtained from their projection onto the cortical surface (Koessler et al., 2009) and converted to MNI space using the Nonlinear Yale MNI to Talairach Conversion Algorithm (Lacadie, Fulbright, Rajeevan,

Constable, & Papademetris, 2008) (F4: x = 42, y = 24, z = 46; F8: x = 55, y= 30, z = -1 ; FP2: x = 24, y= 66, z= 12). Areas outside the brain were removed.

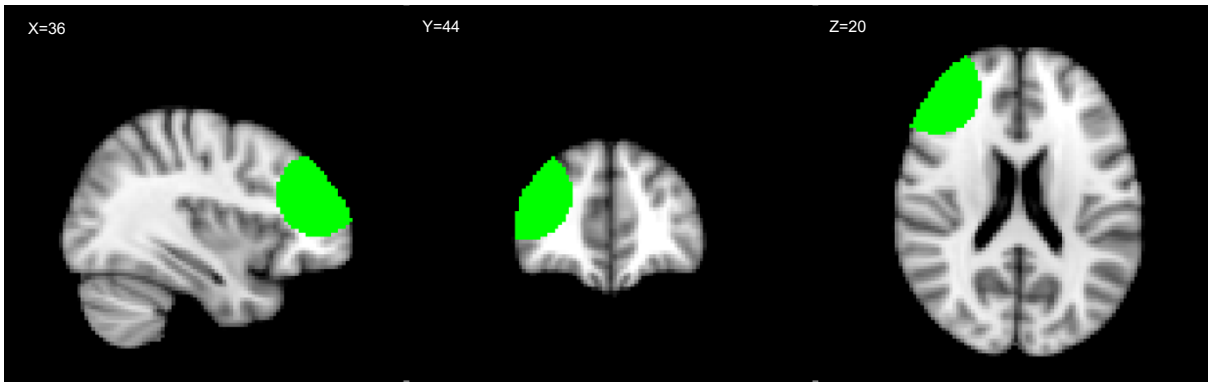


Figure 6.7: MNI brain template with the mask centred around the stimulation target (the right dorsolateral prefrontal cortex) superimposed (in green)

For each participant, mean values of FC strength during real and sham tDCS were extracted for the networks of interest encompassing the mask. Specifically, these RSN were the salience network, the default mode network and the right executive network. Examples of visual overlap between the mask and these networks for healthy younger adults are shown in Figure 6.8.

Extracted network strength was analysed via a series of repeated measures ANOVA, one per network, with Group (younger adults vs older adults vs stroke patients) as a between-subjects factor and Stimulation (real vs sham tDCS) as a within-subjects factor. All main effects and the interaction did not reach statistical significance [all p values >.05] (see Table 6.4 for the main effect of Stimulation).

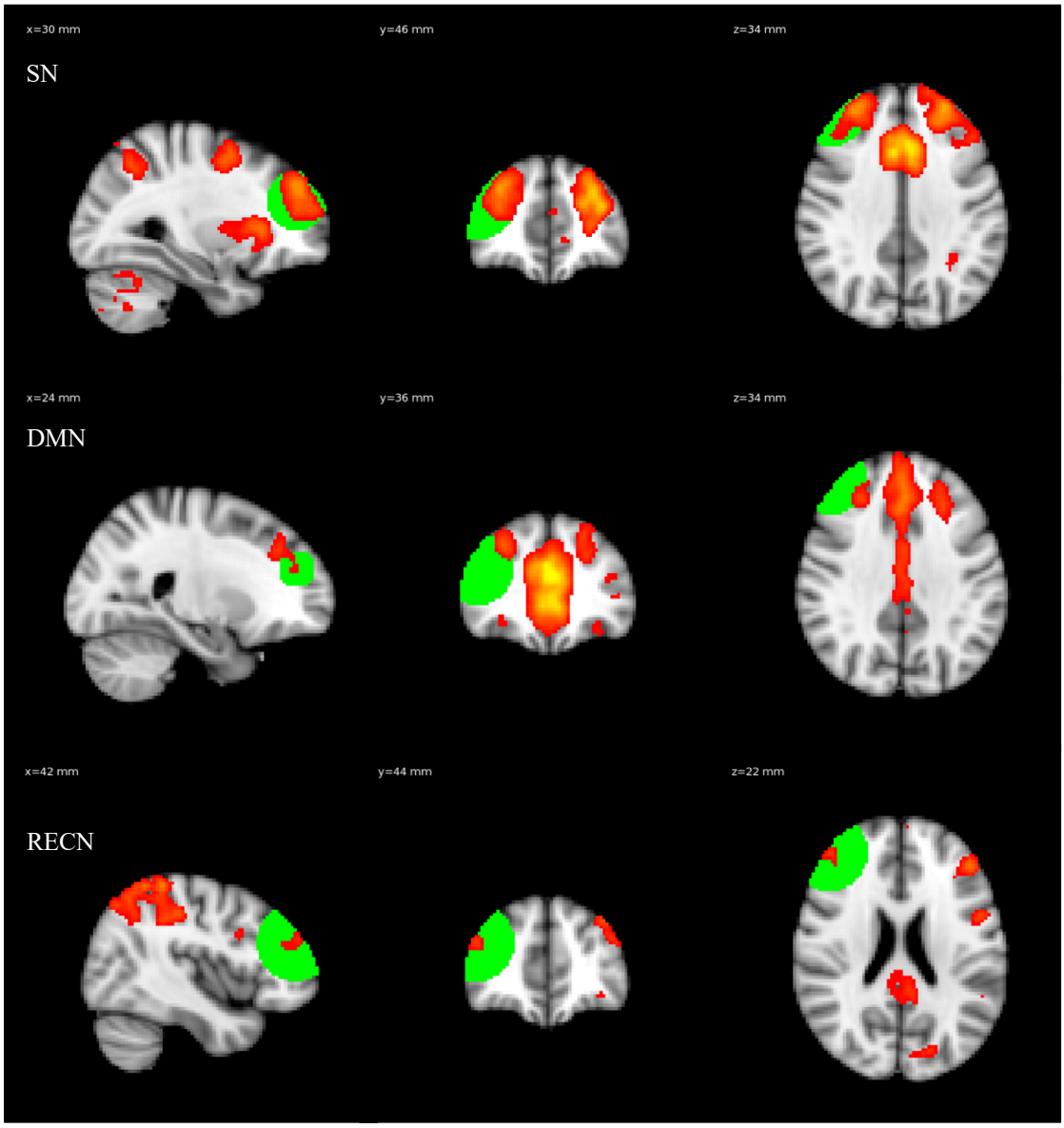


Figure 6.8: Overlap of the Salience Network (SN), Default Mode Network (DMN) and Right Executive Control Networks (RECN) with a mask spanning the stimulation site (in green) for younger adults

Network	Real tDCS	Sham tDCS	p values
Salience	+0.06	+0.01	p=.612
Default Mode	+0.74	+0.68	p=.350
Right Executive Control	+0.65	+0.69	p=.485

Table 6.4. Mean connectivity strength during real/sham tDCS measured within each network for the area overlapping a mask spanning the stimulation site.
Probability values (p) for the main effect of Stimulation are shown.

In sum, no difference in connectivity strength between groups emerged for the area underneath the electrodes. This confirmed that patients included in the study had preserved connectivity in the area targeted by tDCS – in keeping with the exclusion criterion of brain damage in the area targeted by stimulation.

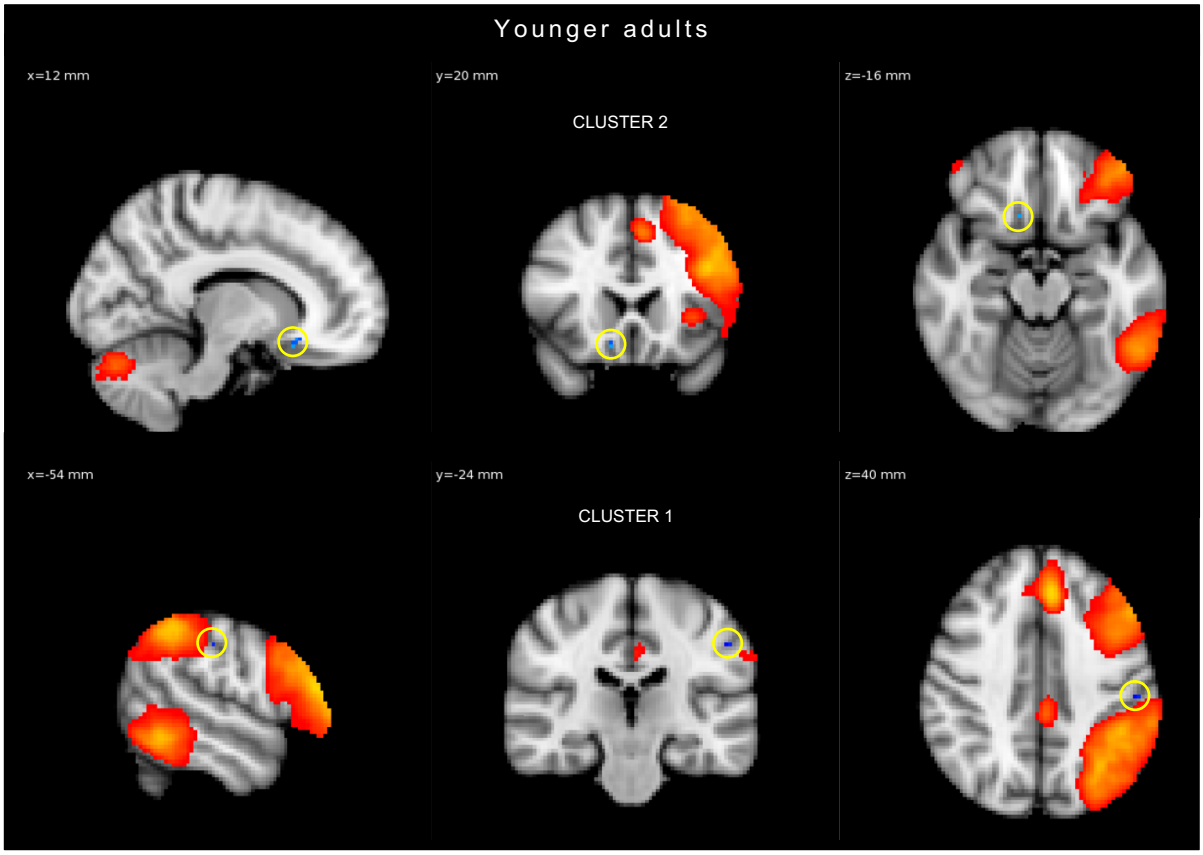
In addition, this analysis showed that the targeted application of tDCS did not induce local changes in functional connectivity: no significant difference in mean connectivity strength was revealed during real, as compared to sham tDCS, for the area underneath the electrodes. Thus, any effects of tDCS are likely to be more widespread, affecting large-scale networks. This possibility was put to test in a series of analyses described in the following sections.

4.3.1.2 Whole-brain functional connectivity during real and sham tDCS

In order to evaluate whether tDCS was able to modulate FC within each RSN of interest, within-subject spatial maps representing the difference in functional connectivity between the two conditions (real minus sham tDCS) were computed for each participant using the subtraction tool in fslmaths. These 'difference' maps were contrasted within each group and between groups, as described below.

Within-group analysis. A series of one-sample t-tests, one per component, were performed on the set of 4D spatial volumes reflecting the difference in connectivity between real and sham tDCS. This allowed to test the hypothesis that the average value of runReal-runSham was different to 0.

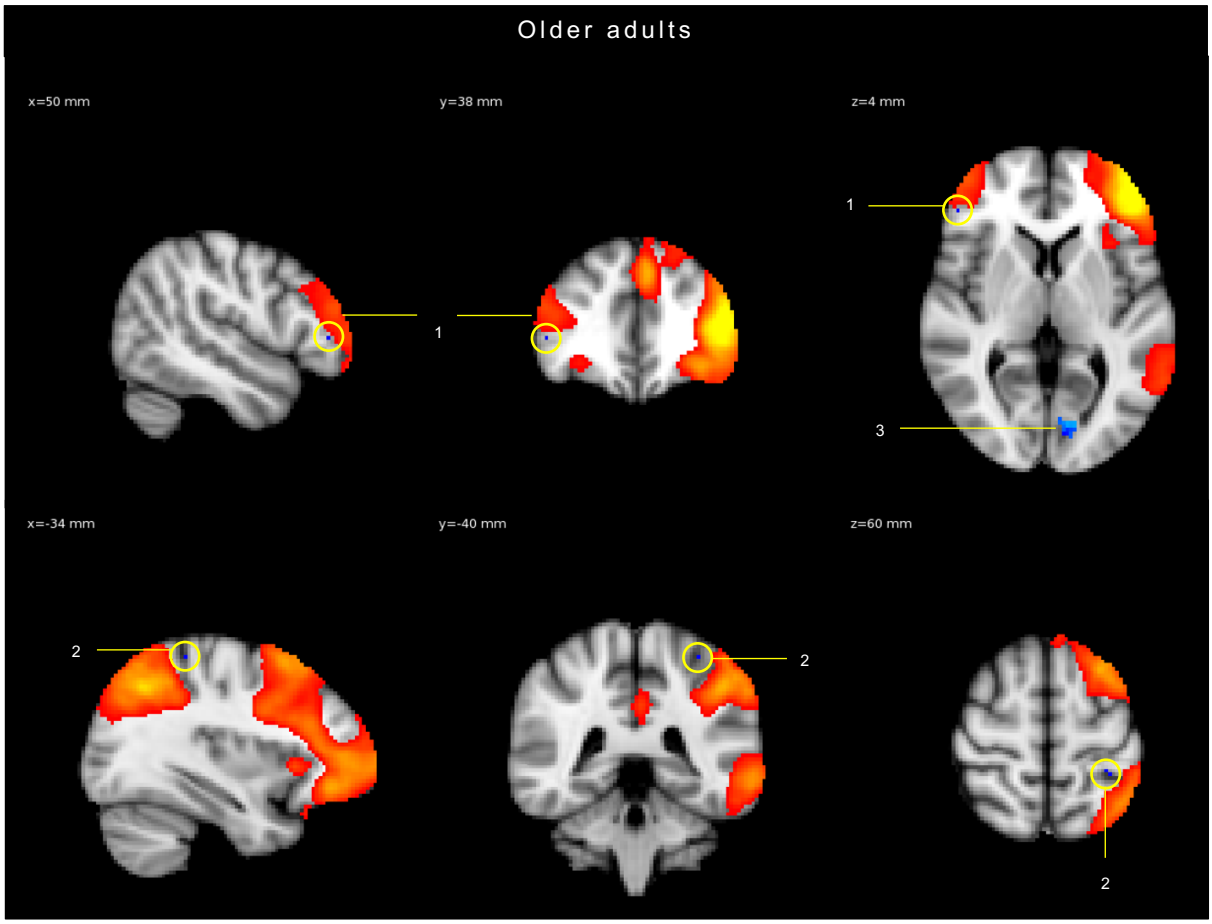
Difference maps were found to be significantly different from zero in the left executive control network (LECN) across all groups [younger adults: p=.018; older adults: p=.009; stroke patients: p=.026]. Small clusters of increased connectivity within LECN were detected during real, as compared to sham tDCS, in different regions across the brain.



LECN clusters	Voxels	X	Y	Z	Region	FC difference
1	2	-54	-24	40	Left supramarginal gyrus	+6.34
2	5	12	20	-16	Right orbito-frontal	+7.76

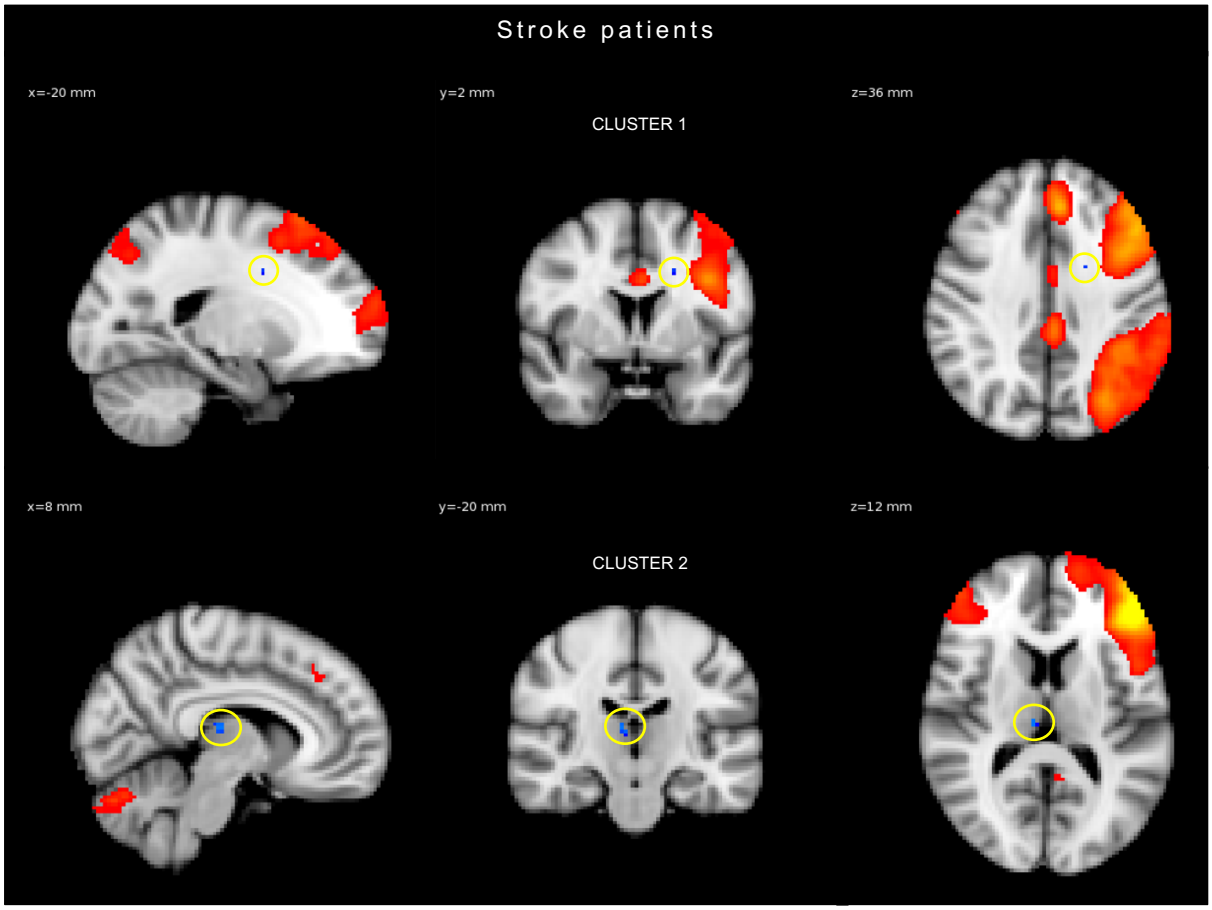
Figure 6.9. Regions showing increased FC during real as compared to sham tDCS within the LECN in healthy younger adults
X, Y and Z refers to MNI coordinates for peak of difference in FC.

In healthy adults, these regions were the right prefrontal and left parieto-occipital cortices (Figures 6.9 and 6.10). In stroke patients, clusters were identified in the right thalamus and in the left corona radiata (Figure 6.11). The connectivity of these areas with the main regions of the LECN was different in the two conditions. Specifically, in all the identified voxels, functional connectivity was higher during real as compared to sham stimulation. This result means that, even if on average these areas are not strongly connected with regions within the LECN (i.e., they fall 'outside' the group average network displayed in Figure 6.6), these regions showed increased connectivity with other regions within that network during real tDCS, as compared to sham tDCS.



LECN clusters	Voxels	X	Y	Z	Region	FC difference
1	1	50	38	4	Right inf frontal gyrus	+6.96
2	2	-34	-40	60	Left postcentral gyrus	+4.51
3	92	-12	-86	2	Left occipital lobe	+5.16

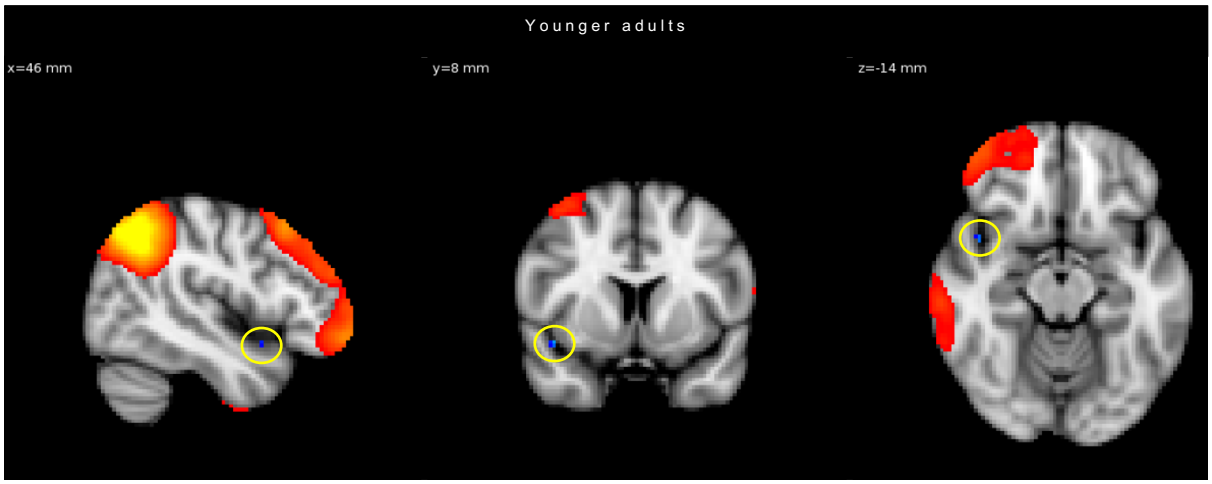
Figure 6.10. Regions showing increased FC during real as compared to sham tDCS within the LECN in healthy older adults
X, Y and Z refers to MNI coordinates for peak of difference in FC.



LECN clusters	Voxels	X	Y	Z	Region	FC difference
1	2	-20	2	36	Left sup corona radiata	+5.43
2	13	8	-20	12	Right thalamus	+10.09

Figure 6.11. Regions showing increased FC during real as compared to sham tDCS within the LECN in stroke patients
X, Y and Z refers to MNI coordinates for peak of difference in FC.

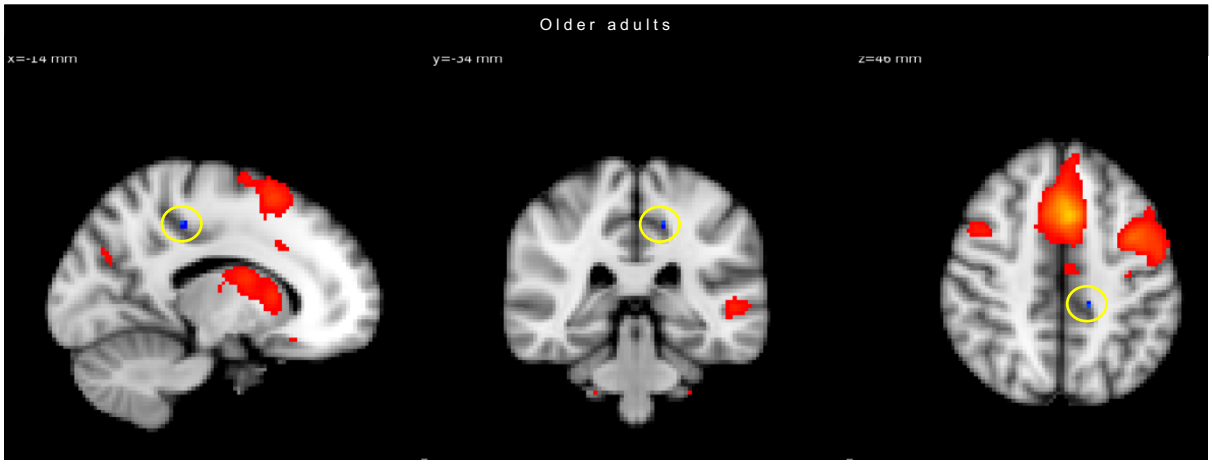
For the younger group only, an increase in connectivity during real tDCS, as compared to sham, also emerged in the right-executive control network [p=.019]. A small cluster of significant voxels was found in the right superior temporal pole (Figure 6.12).



RECN clusters	Voxels	X	Y	Z	Region	FC difference
1	6	46	8	-14	Right superior temporal	+18.48

Figure 6.12. Regions showing increased FC during real as compared to sham tDCS within the RECN in younger healthy adults
X, Y and Z refers to MNI coordinates for peak of difference in FC.

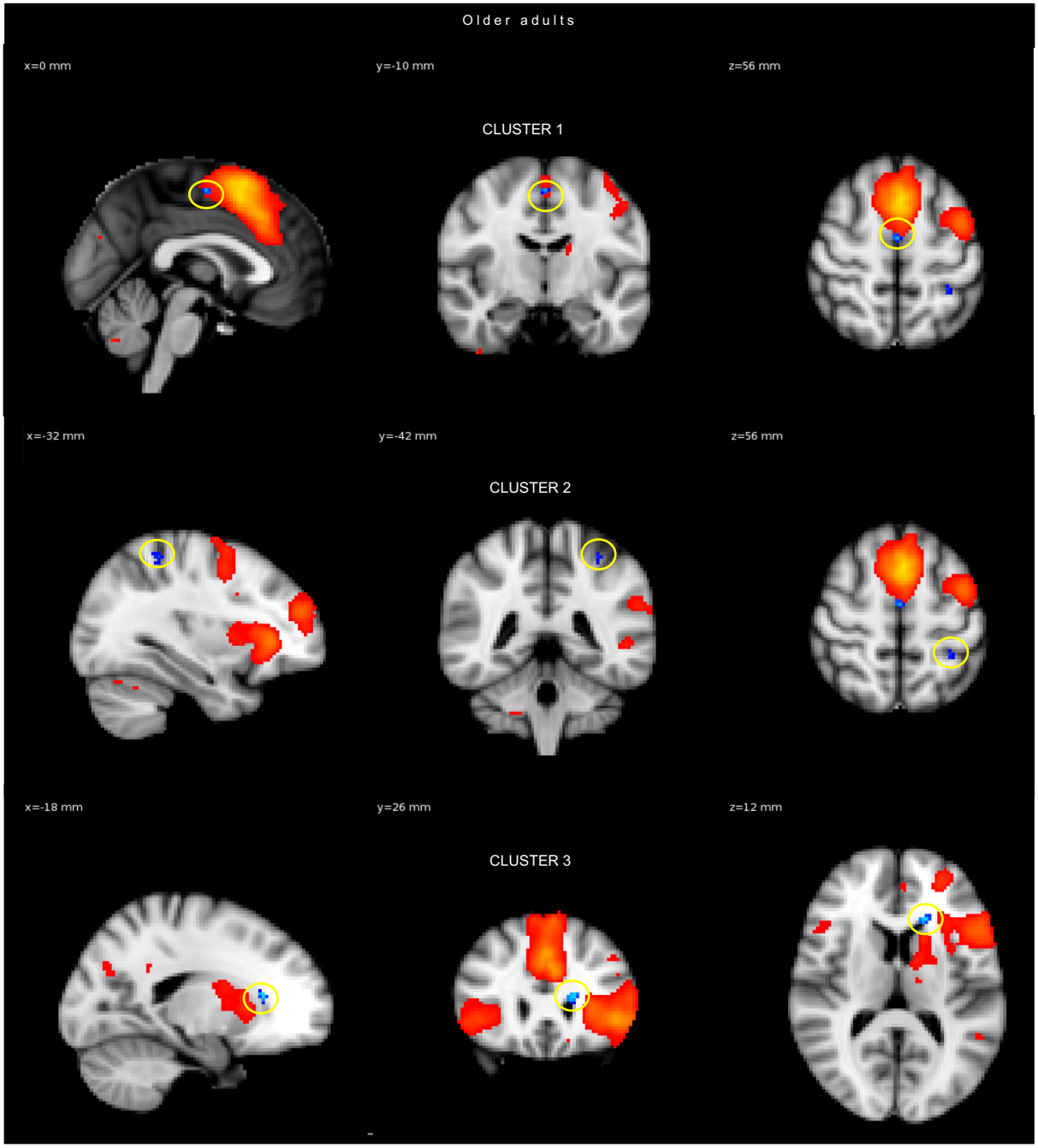
In the group of older adults only, an increase in connectivity was found during real, as compared to sham tDCS, within the salience network and the visuospatial network [respectively, p=.034 and p=.008] (Figures 6.12 and 6.13).
In particular, it was the left mid cingulum that showed increased connectivity with the main areas within the salience network (Figure 6.13).



SN clusters	Voxels	X	Y	Z	Region	FC difference
1	5	-14	-34	46	Left mid cingulum	+6.38

Figure 6.13: Regions showing increased FC during real as compared to sham tDCS within the salience network in older adults
X, Y and Z refers to MNI coordinates for peak of difference in FC.

Within the visuospatial network, significant clusters of increased FC during real tDCS as compared to sham were identified in the right supplementary motor area and in the left superior frontal gyrus and postcentral gyrus (Figure 6.14).



VSN clusters	Voxels	X	Y	Z	Region	FC difference
1	10	0	-10	56	Right suppl motor area	+8.17
2	11	-32	-42	56	Left postcentral gyrus	+5.55
3	28	-18	26	12	Left sup frontal gyrus	+6.41

Figure 6.14. Regions showing increased FC during real as compared to sham tDCS within the Visuospatial network (VSN) in older adults
X, Y and Z refers to MNI coordinates for peak of difference in FC.

Between group comparisons. The maps representing paired differences in functional connectivity during real and sham stimulation conditions were compared between groups using two-samples unpaired t-tests. No difference in FC in response to tDCS emerged for any of the five networks of interest between younger and older adults and between older adults and stroke patients [all p values >.05].

4.3.1.3 Control for a potential confound

A control analysis was performed in all healthy participants to confirm that the two sham applications were comparable, regardless of whether sham stimulation preceded or followed real tDCS. In other words, I examined, for the group who received real stimulation first, whether there was any carry-over of the effect of stimulation on connectivity that could contaminate the sham fMRI run. Unpaired t-tests were used to compare FC, within each network, between two groups of healthy participants: those who received real first (n=18) and those who received sham first (n=22). No significant difference was revealed for any of the networks of interest [all p values >.05].

4.3.1.4 Intrinsic brain network connectivity during real and sham tDCS

A new ICA that included all participants (n=56) was performed, followed by the Dual Regression approach to identify individual contributions to the group average. Mean connectivity strength within the 5 networks of interest was interrogated by extracting the BOLD time series for voxels within each network.

To do so, the output z-maps of dual regression, which reflect subject- and condition-specific strength of FC for each network, were masked by the group average maps of the respective component, and mean values were extracted for each subject. These were used as an indicator of the strength of FC within each network of interest, as per previous relevant works in the field (Amadi, Ilie, Johansen-Berg, & Stagg, 2014; Antonenko et al., 2017; Bachtiar et al., 2015; Binnewijzend et al., 2012; Rinne et al., 2018).

Connectivity strength during real and sham tDCS for each participant was then contrasted by means of a series of repeated measures ANOVA with Group (younger adults vs older adults vs stroke patients) as a between-subjects factor and Stimulation (real vs sham tDCS) as a within-subject factor. Bonferroni corrected post-hoc tests were performed to explore significant effects.

A main effect of Stimulation emerged for the left executive control network (LECN) [$F(1,54)=5.232$, $p=.026$, $\eta_p^2=.088$], with increased connectivity strength observed during real (mean +4.57) as compared to sham tDCS (mean +4.32) (Figure 6.15). For all other networks of interest, mean connectivity strength was not significantly different during real vs sham tDCS [all p values >.05] (Table 6.5).

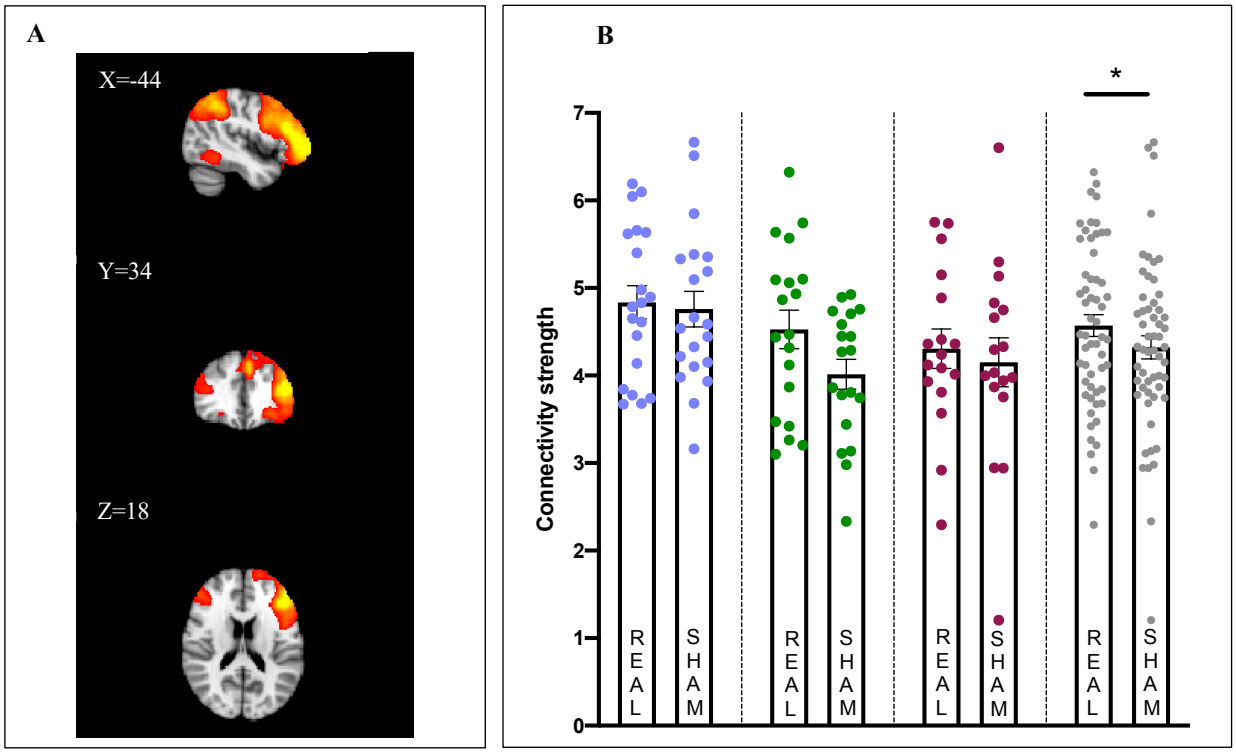


Figure 6.15. Group mean Left Executive Control Network (LECN) [panel A]; individual FC strength observed within the LECN during real and sham tDCS [panel B]
Panel A -The mean LECN was derived from resting state fMRI connectivity analysis using Independent Component Analysis (ICA) for all 56 participants across the 2 stimulation conditions; Panel B – The main effect of stimulation is shown, with increased connectivity observed during real tDCS as compared to sham tDCS, across all groups (grey dots). Individual datapoints are also shown for younger (orchid dots), older (green dots) and stroke patients (maroon dots) separately. Black bars represent mean and SEM.

Network	Real tDCS	Sham	STIMULATION	GROUP	S x G
SN	+3.37	+3.46	p=.495	**p=.000**	p=.955
DMN	+4.57	+4.50	p=.634	**p=.000**	p=.652
LECN	**+4.57**	**+4.32**	**p=.026***	p=.042[1]	p=.125
RECN	+4.19	+4.21	p=.791	p=.079	p=.875
VSN	+3.97	+3.37	p=.076	**p=.021**	p=.447

Table 6.5. Functional connectivity strength for each network during real and sham tDCS
Network strength was derived using the group average as a mask.

In addition, significant differences in mean connectivity between groups were revealed.

For the Salience network, a main effect of Group emerged [$F(2,54)=10.788$, $p=.000$, $\eta_p^2=.285$] (Table 6.5). Mean connectivity was higher in younger (mean +3.71) and older (mean +3.65) adults, as compared to stroke patients (mean +2.87) [respectively p=.000 and p=.001].

For the DMN, a main effect of Group emerged [$F(2,54)=20.484$, $p=.000$, $\eta_p^2=.431$]. Networks strength within this network was significantly different between younger adults (mean +5.28) and older adults (mean +4.40) [p=.001], and between younger adults and stroke patients (mean +3.82) [p=.000].

For the Visuospatial network, a main effect of Group emerged [$F(2,54)=4.146$, $p=.021$]. Connectivity strength within the network was significantly different between younger adults (mean +4.18) and stroke patients (mean +3.60) [p=.018]; mean connectivity for older adults (mean +4.09) was not significantly different to that of other groups (all p values >.05).

For the LECN, a main effect of Group emerged [$F(2,54)=3.362$, $p=.042$, $\eta_p^2=.111$]. However, between groups comparisons did not survive the Bonferroni correction.

[1] Between groups pairwise comparisons did not survive Bonferroni correction.

For the RECN, the main effect of Group was not significant [F(2,54)=2.668, p=.079].

Importantly, these differences between groups were not specific for the real or the sham tDCS condition. For all five RSN of interest, the interaction between Stimulation x Group was not significant [all p values >.05] (Table 6.5). This finding confirmed that the modulation exerted by tDCS on the LECN described above was present across all groups, with no significant difference between groups in response to brain stimulation.

This analysis was then repeated using an independent mask derived from a well-characterised anatomical atlas, instead of using the group average as a mask, to ensure that the effect described above was not driven by any intrinsic characteristic of the population that was tested. For this confirmatory analysis, the output of Dual Regression was masked using the networks from the Shirer atlas (Shirer et al., 2011), and a series of repeated measures ANOVA with Group (younger adults vs older adults vs stroke patients) as a between-subjects factor and Stimulation (real vs sham tDCS) as a within-subjects factor was conducted. Results paralleled those described above, with a significant main effect of Stimulation observed for the left executive control network (LECN) across groups [F(1,53)=4.523, p=.038, η_p^2=.079] (Table 6.6).

Network	Real tDCS	Sham tDCS	STIMULATION	GROUP	S x G
SN	+1.84	+1.95	p=.138	**p=.001**	p=.968
DMN	+3.27	+3.20	p=.532	**p=.000**	p=.785
LECN	**+3.63**	**+3.44**	**p=.038***	p=.301	p=.204
RECN	+2.17	+2.17	p=.892	**p=.027**	p=.693
VSN	+2.59	+2.48	p=.235	**p=.004**	p=.255

Table 6.6. Functional connectivity strength for each network during real and sham tDCS
Network strength was derived applying a mask derived from a robust anatomical atlas.

In sum, this analysis confirmed the results that emerged from the previous analysis that utilised a sample specific mask.

4.3.2 Behaviour:connectivity analysis

A second question relates to the relationship between functional connectivity changes and behavioural changes. This analysis was performed in the subset of participants (healthy younger, older adults and stroke patients) who took part in both the imaging experiment described in this Chapter and the crossover behavioural task described in Chapters IV and V. Out of 18 patients who took part in both studies, four had to be excluded because they did not meet censoring criteria set for movement. The final cohort of participants for this analysis therefore included three groups with the following sizes: younger adults (n=13), older adults (n=8) and stroke patients (n=14).

In order to relate tDCS-induced effects on performance to connectivity changes, a series of Pearson's correlations (two-tailed) were performed using as variables of interest the delta in performance and the delta in network strength. Specifically, changes in accuracy during brain stimulation (computed as real minus sham) were related to changes in functional connectivity during brain stimulation (computed as real minus sham).

Three Independent Component Analysis (one per group) were run. Difference maps (computed as real_run minus sham_run) reflecting changes in network connectivity between real and sham tDCS were computed. Mean functional connectivity values were extracted for each network using the anatomical mask derived from a robust anatomical atlas (Shirer et al., 2011). Network strength was then correlated to difference in task accuracy (also computed as real_score minus sham_score).

No significant correlations emerged for healthy younger and older adults [all p values >.05]. However, in the group that showed the biggest behavioural changes and the biggest variation in behavioural change, i..e., the patient group, changes in performance were found to be significantly and strongly correlated with changes in activation within the default mode network (DMN) [r=-.452, p(two-tailed)=.006] and the right executive control network (RECN) [r=+.496, p(two-tailed)=.002].

Thus, changes in network strength were found to be related to benefits on task performance in the patient group. Behavioural response to tDCS was found to be associated with decreased FC within the default mode network and increased FC within the right executive control network in response to the application of brain stimulation.

4.3.3 tDCS side effects

Any potential side-effects related to the application of tDCS was monitored via interphone immediately after each fMRI run using a short questionnaire adapted for in-scanner administration (Appendix IX). Participants were asked to state whether they thought they received any stimulation or not, and to rate the intensity of six potential side-effects on a scale from 0 (absent) to 4 (unbearable). Data were available for 20 younger adults, 21 older adults and 20 stroke patients.

4.3.3.1 Perception of stimulation

A first analysis aimed to establish whether active and sham targeted tDCS were undistinguishable when delivered in the scanner during fMRI acquisition. To relate the two categorical variables, 'stimulation delivery' (yes vs no) and 'stimulation perception' (yes vs no), a series of chi-square tests of independence was performed to examine the relationship between the delivery and the perception of brain stimulation (Figure 6.16).

In younger adults, the relation between these variables was significant [$\chi^2(1)=7.02$, p =.008]: 67% of them correctly identified the stimulation condition. A tendency to overreport the delivery of stimulation was also noted: when questioned, they reported the sensation of stimulation on 77% of occasions, even though this was actually delivered 50% of the times in a cross-over fashion.

For older adults, the association between delivery and perception of stimulation was not significant [$\chi^2(1)=2.78$, p =.095]. The stimulation condition was correctly

identified 62% of the time, and in 69% of cases they stated that they had received stimulation.

Perception of stimulation was also at chance level for stroke patients [$\chi^2(1)=0.14$, p =.705]: they correctly identified the stimulation condition 52% of the time. Only one patient was able to correctly identify both real and sham tDCS delivered intra-scanner. Patients seemed to overreport the delivery of stimulation (77%).

tDCS DELIVERY	tDCS PERCEPTION	
Younger	YES	NO
YES	19	1
NO	12	8

tDCS DELIVERY	tDCS PERCEPTION	
Older	YES	NO
YES	17	4
NO	12	9

tDCS DELIVERY	tDCS PERCEPTION	
Stroke	YES	NO
YES	16	4
NO	15	5

Figure 6.16. Awareness of stimulation for the three groups of participants
The contingency tables show, for each group, participants' awareness of the brain stimulation delivered intra-scanner. The percentage of participants who correctly guessed the tDCS condition they had been exposed to was 67% (younger adults), 62% (older adults) and 52% (stroke patients). Note that all groups tended to overreport the presence of stimulation.

After the familiarisation phase outside the scanner, all participants were made aware that they may or may not receive stimulation inside the scanner, and that they were going to be asked about it. Whereas in principle they may have spent their 'rest' thinking about whether they were receiving or not any stimulation, analyses of their responses suggest that the vast majority of participants was not able to discriminate between real and sham: older adults and stroke patients were at chance level when asked to indicate the allocation group.

4.3.3.2 Intensity of tDCS side-effects

Itchiness, burning and tingling sensations were the most severe side-effects reported by study participants following active stimulation; on average, they were reported as very mild sensations (respectively, 0.52/4, 0.75/4 and 0.75/4).
A series of repeated measures ANOVA (one per potential side-effect) with Group (younger vs older vs stroke) as a between-subject factor and Stimulation (real vs

sham tDCS) as within-subjects factor was conducted, including the intensity of each sensations as dependant variable. The mean severity of each potential side-effects for all participants is reported in Table 6.7.

Side-effects	Active tDCS	Sham tDCS	p value
Tingling	+0.75	+0.51	**p=.007**
Itching	+0.52	+0.33	**p=.011**
Pain	+0.13	+0.05	**p=.019**
Burning	+0.75	+0.59	p=.075
Metallic taste	+0.02	+0.02	p=1
Anxiety	+0.03	+0.02	p=.337

Table 6.7. Mean adverse effects reported by all subjects after real and sham tDCS
Numbers corresponds to the mean severity of each side-effect. P values are derived from a series of repeated-measures ANOVA.

Tingling was reported as more severe following real tDCS as compared to sham tDCS across all groups [$F(1,58)=7.805$, $p=.007$, $\eta_p^2=.119$] - no main effect of Group or interaction Group x Stimulation emerged [all p values >.05].

Itching was also more intense after real as compared to sham tDCS [$F(1,58)=6.947$, $p=.011$, $\eta_p^2=.107$], with the interaction Condition x Group at significance levels [$F(2,58)=3.084$, $p=.053$, $\eta_p^2=.096$] (Figure 6.17). Bonferroni corrected post-hoc tests, used to explore this interaction, revealed that itching was reported as more intense after real as compared to sham tDCS by the younger group only (respectively, +0.850 and +0.450) [p=.003]. Also, younger adults reported itching more intensely than stroke patients following the application of real tDCS (respectively, +0.850 and +0.300) [p=.045]. All other pairwise comparisons were not statistically significant [all p values >.05].

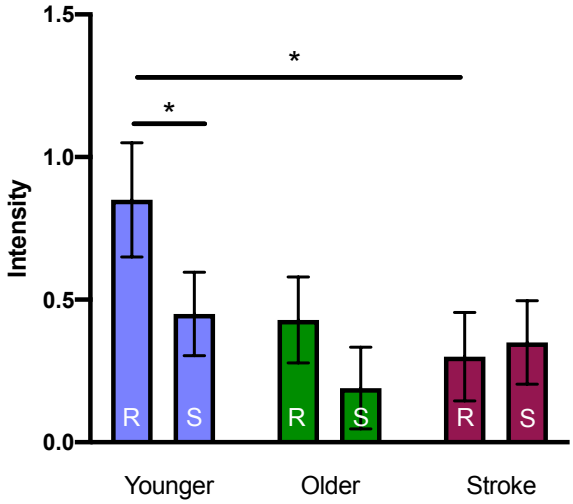


Figure 6.17. Intensity of itching sensation as reported by participants after real (R) and sham (S) tDCS

Similarly, real tDCS was reported as being more painful when compared to sham tDCS [$F(1,58)=5.802$, $p=.019$, $\eta_p^2=.091$], with the interaction Condition x Group at significance levels [$F(2,58)=3.057$, $p=.055$, $\eta_p^2=.095$]. Post-hoc tests exploring this interaction were performed; any difference in the perception of pain between groups and condition did not survive the Bonferroni correction [all p values >.05].

No significant difference between real and sham tDCS in the reported intensity of burning sensation, metallic taste and anxiety emerged [all p values >.05]; the interaction between Group x Stimulation was also not significant for these sensations [all p values >.05].

The main effect of Group systematically emerged as non-significant for all side-effects [all p values >.05], suggesting that all groups were equally able to rate the intensity of any perceived change.

In sum, general discomfort was very low: the intensity of subjective side effects was, on average, always below 1 on a scale from 0 to 4, with 0 being absent and 1 being a mild side-effect. Burning, a metallic taste, and anxiety were judged just as intense after real or sham tDCS. Subjective tingling and pain were, on average, more intense following real as compared to sham tDCS across all groups. Itching

was more severe following real as compared to sham tDCS, particularly for the younger group who seemed to perceive more sensations on the skin than stroke patients. This may be why younger adults responded above chance when asked directly whether they thought they had just received brain stimulation or not - older participants and stroke patients were both at chance level. All participants showed a tendency to overreport the presence of stimulation.

5 Ad interim discussion

This study aimed to examine synchronisation of neural activity, as measured with resting-state fMRI, during targeted tDCS administration. Specifically, functional connectivity during real and sham tDCS was measured in adults spanning a wide age range (20-80 years old) and in patients affected by right hemispheric stroke.

5.1 tDCS-induced changes in FC

A primary analysis explored changes in resting-state connectivity induced by the application of brain stimulation, by comparing real and sham tDCS conditions.

A network analysis, restricted to the region below the electrodes, revealed a lack of local changes in connectivity strength induced by tDCS. This finding may indicate that the modulatory effect of tDCS may be transmitted from the directly stimulated brain regions to other distant, but functionally connected areas.

A recent study in a non-humate primate model showed that tDCS induced local and widespread changes in connectivity (Krause et al., 2017). Local and remote effects have been also studied using a multimodal imaging approach, similar to the one used in the present study, which showed that tDCS applied to a key node produced effects in remote but connected brain areas (Li et al., 2019). Other researchers also showed that, using transcranial alternating current stimulation, the effects of stimulation were not at their strongest in the area below the electrodes, but rather in areas distant from the electrodes (Cabral-Calderin et al., 2016).

For the delivery of brain stimulation, all the studies mentioned above utilised traditional large pads positioned over the two hemispheres. The present work confirms and extends these findings, having utilised a more targeted approach to increase focality and constrain the current delivery to a circumscribed region within the right prefrontal cortex. Interestingly, despite the targeted montage, the modulatory effects of the application of brain stimulation were not local.

This investigation indeed revealed that prefrontal tDCS modulated whole-brain functional connectivity. A consistent and robust response to tDCS emerged for the left executive control network (LECN) across all groups. Within this network, it was possible to identify small clusters of brain regions in both hemispheres that showed increased mean functional connectivity during real as compared to sham tDCS. No area showed decreased FC during stimulation vs Sham tDCS.
Results from the analysis contrasting mean connectivity strength between real and sham tDCS pointed in the same direction, with higher mean strength connectivity found during real as compared to sham tDCS within the LECN - this result was arrived at using two different masking methods.

In sum, the targeted application of tDCS seems to have influenced activity within a large-scale distributed brain network. These effects on network connectivity were mainly registered in the hemisphere contralateral to the stimulation in all groups. Stimulation induced the same pattern of response in all groups – patients were selected depending on the integrity of the stimulation target (the right DLPFC).

Evidence from the resting state fMRI literature suggests that electrical stimulation of the DLPFC may significantly affect resting functional connectivity in both hemispheres. For instance, anodal stimulation of the right DLPFC has been shown to modulate thalamocortical networks bilaterally (Sankarasubramanian et al., 2017). Another study, which targeted the left DLPFC, demonstrated post-stimulation changes in large-scale patterns of resting-state connectivity both close to the anode and cathode stimulation sites and in distant brain regions bilaterally (Keeser et al., 2011). A study by Park and colleagues showed that functional

connectivity of the stimulated region (the left DLPFC) with areas within the ipsilateral hemisphere was increased, and connectivity with areas within the contralateral hemisphere was decreased (Park et al., 2013).
An important difference between these studies and the present research is that these studies all measured resting state functional connectivity following (vs during) the application of tDCS, which was delivered outside the scanner using conventional large tDCS electrodes.

In addition to the effect within the LECN found across groups, within-group analysis also showed tDCS-induced increase in FC in the right executive control network for healthy younger adults, and within the salience and visuo-spatial networks for older adults. However, when directly comparing groups, no systematic difference in network response to tDCS was observed. This was arrived at via two different analyses, the whole-brain FC analysis and the intrinsic network strength analysis, which both showed that the effects of tDCS were not specific to one group. Instead, groups showed, on average, similar potential to respond to tDCS. Older adults, and patients whose lesions did not encroach the area underneath the electrodes, proved to be able to respond to the application as much as younger individuals.

5.2 Behaviour:connectivity relationship

Further analysis aimed at revealing possible links between resting state networks and behavioural response to tDCS. The present study indicated that stroke patients who demonstrated greater behavioural benefit in response to tDCS also experienced a greater increase in connectivity within the right executive control network in response to tDCS, in parallel with reduced response of the default mode network.

This association between behavioural improvement and change in network strength emerged in the patient group only, most likely because this group had bigger potential for improvement on this task and greater variability in improvement. This clinical population was indeed affected by attentional deficits,

and the task was chosen to detect this. Therefore, future studies wishing to explore whether this finding also holds in healthy populations, may wish to employ a task on which healthy adults are further away from ceiling.

5.3 Study limitations and future directions

A first consideration in discussing the limitations of this work is that the concept of resting state is by definition ambiguous, as cognitive functions 'at rest' are not controlled for (González-Hernández et al., 2005; Stark & Squire, 2001). However, differences in mental states are particularly problematic for experiments comparing task activity with activity at rest. In this study, each participant was assigned to a treatment sequence (real-sham or sham-real), receiving two sessions of fMRI at rest - the use of a crossover design mitigates against this potential confound. Clear and consistent instructions were given to every participant before each run; they were then briefed afterwards to ensure that none fell asleep during the stimulation interval.

Motion may also represent a confound in fMRI studies, and a major factor of exclusion especially in clinical populations who may struggle to tolerate long scanner times. To mitigate for this factor, special attention was paid in the acquisition phase: positioning/padding around was optimised, breaks were allowed after each sequence and a reflective headset was offered to allow participants watch soothing videos during anatomical scans. In the data processing phase, only a small proportion of data were discarded as they did not pass the criteria for motion data quality. For a thorough discussion of the potential and limitations of the resting-state fMRI approach, see the review by Buckner and colleagues (Buckner, Krienen, & Yeo, 2013).

A limitation of the study is that the interactions between networks, which might also change in response to the application of tDCS, were not evaluated. Rather, in the present experiment, the focus was on connectivity within specific resting-state networks of interest in response to tDCS application. Future studies should address the relationship across different networks, such as the interplay between

the LECN and the RECN. Also, considering that vigilant performance has been found to be associated with smooth DMN activation coupled with activation of dorsal-attention and salience network regions (Kucyi, Hove, Esterman, Hutchison, & Valera, 2016), future studies looking at the coupling of these networks in older and clinical populations would be valuable.

An additional limitation is that, in the present study, a mean value was taken for each 10-minute run, under the assumption that connectivity is static throughout the fMRI examination. Future studies could examine the temporal dynamics of the effects of tDCS on brain networks examining time-varying functional connectivity (Hutchison et al., 2013). This might be achieved for instance by dividing the acquisition time into smaller temporal segments, and then comparing changes in functional connectivity across time. This analysis may provide insights into the network properties in the healthy and damaged brain, and help characterise the effects induced by HD-tDCS. For instance, this may show whether, on average and at the individual level, changes over time follow a linear or non-linear relationship.

This work was able to demonstrate, at the group level, an association between changes in vigilant performance and resting state connectivity in response to tDCS in a sample of stroke patients. Further research is however needed to clarify whether a potential link also exists in healthy individuals across the lifespan. A longer task (e.g., Nelson, McKinley, Golob, Warm, & Parasuraman, 2014), or a task monitoring moment-to-moment ability to stay on task (e.g., Petton et al., 2019), may be more suitable to track fluctuations in performance in a sample of healthy participants.

Linked to the previous point, another limitation of the current work is that the inferences that can be drawn from the study are restricted to the group-level, rather than the individual level. Patients showing, on average, higher functional connectivity within the visuospatial network in response to the application of tDCS, also showed increased behavioural response. This is not sufficient to allow for the accurate prediction of connectivity-behaviour relationship in each patient,

which would greatly facilitate the clinical translation of these findings. A possible expansion of this would be to use neuroadaptive paradigms and simulation approaches, to tailor task characteristics and stimulation parameters to brain anatomy and pathology (e.g., see Lorenz et al., 2016).

6 Conclusions

Although the potential of brain stimulation has been questioned by some (e.g., see Horvath, Forte, & Carter, 2015; Minarik et al., 2016), the majority of the available evidence suggests that careful study of stimulation approaches is justified. The work presented here supports the notion that there is scope for future research dissecting how tDCS modulates behaviour in healthy and clinical populations (Filmer, Mattingley, & Dux, 2019).

In the first part of this chapter, lesion-mapping methodology was used to gain an insight into the neural correlates of vigilant performance and tDCS responsiveness. Brain damage centred on the right corpus callosum and the right cingulum was associated with worse performance on the vigilance task.

The lesion anatomy approach was also useful in identifying key factors that interact with tDCS effects, providing insight into the well-known interindividual variability in response to tDCS. At the single-patient level, tDCS modulations of behaviour varied between individuals (see Chapter V, Section 4.1.3), with individuals affected by lesions involving the right thalamus and the right postcentral gyrus being less likely to benefit from tDCS over the right dorsolateral prefrontal cortex.

Here, two voxelwise techniques have been used to relate behaviour and lesion location. Each method carries limitations, which have implications for how we interpret the results.

The first method is the lesion subtraction analysis, which is a descriptive method: the output is a map of areas more frequently damaged in patients who did not show a response to tDCS. My interpretation consisted in a cautious description of these loci, with the use of a brain atlas for localisation purposes. A larger patient study examining anatomical correlates of tDCS response is needed to confirm these preliminary findings using a mass-univariate approach, which performs many statistical tests, at the voxel level.
When I used the second approach, the univariate statistical comparison method, result maps were corrected using permutation-based thresholding, with a large number of permutations. This correction has been recommended in a practical guide prepared by leading experts in neuroimaging, because it offers the same control as the Bonferroni correction when performing a huge number of statistical tests, but has better statistical power in situations where individual voxels are not truly independent (Karnath et al., 2019). When using this approach, a limitation regards the chosen atlas, which is not a trivial issue. The AALCAT atlas was chosen for this study, because it encompassed grey and white matter. Future studies should aim to demonstrate between-atlases concordance.

Another useful approach informing researchers about the mechanisms underlying the effects of brain stimulation is a multimodal combination of techniques. In a separate experiment, conducted in healthy and clinical populations, brain network response during tDCS application was examined. This allowed probing whether improvements in cognitive function correlated with the size of network changes during tDCS. This study showed that, with the specific set of HD-tDCS parameters used here, and electrodes clustered around the right DLPFC, connectivity was significantly increased across groups within the left executive control network during real as compared to sham tDCS. During active tDCS, resting state functional coupling was found to be increased in the contralateral hemisphere in all groups, indicating augmented efficiency in large-scale brain network functioning.

Significant baseline differences between groups in baseline FC were observed in resting state networks tapping into the right hemisphere. However, age and pathology-related differences in network integrity did not seem to affect network response to tDCS, with all groups showing, on average, potential to respond to the application of tDCS.

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Chapter VII:
General discussion

1 Key findings

This thesis focused on investigating the efficacy of non-invasive brain stimulation to improve vigilant attention across the life span and after brain injury. The work was primarily motivated by the potential clinical translation of a treatment involving the application of transcranial Direct Current Stimulation (tDCS) for patients with attentional impairments. Following right-hemispheric stroke, patients suffer from severe attentional problems, for which different experimental interventions have been tried with partial success. At present, there are no convincing randomised trials demonstrating clinically significant improvements. The main problems with evaluating any treatment for neglect are patient variability in presentation and in response to intervention, and the difficulty of observing a therapeutic effect in a recovering population. These issues mandate careful and robust investigation of any potential treatment in this patient group.

Critically, the lateralised attention deficit of neglect has frequently been the key modifiable target of the majority of these treatment studies in this population, following on from its striking manifestations (Stone et al., 1991). However, a generalised difficulty in maintaining attention that is irrespective of the position of a stimulus in space is also part of the syndrome, and may represent a potential target for interventions. In this scenario, neuromodulation approaches such as the non-invasive delivery of electrical brain stimulation have the advantage of being safe, relatively cheap and portable, with the potential to be implemented at home. In addition, tDCS could be set up for delivery at rest (thus being a potential option even in the most severe cases) or as an adjunctive therapy, thereby potentiating other therapies.

In this thesis, I have presented a systematic behavioural and imaging investigation evaluating such an approach, and have demonstrated that the targeted stimulation of the right prefrontal cortex is linked to an improvement in target detection on an attentional task that does not contain a lateralised component. This was demonstrated in a population of individuals with a brain injury as well as in healthy individuals across the lifespan. In addition, I examined the anatomical and functional correlates of response to the targeted delivery of tDCS. Each chapter's contribution is discussed below, followed by a discussion on strengths and limitation of the approach as well as suggestions for future directions in research.

The first Chapter presents an overview of previous research into neglect following right-hemispheric stroke. I explain that neglect is widely regarded as a heterogeneous condition, spanning lateralised and non-lateralised attention deficits. The lateralised component of the syndrome refers to the well-researched difficulty in paying attention to the left-hand side of the space; the non-lateralised component, on the other hand, refers to attentional difficulties that are not necessarily related to the position of a stimulus in space. An example would be the vigilant attention impairment, which manifests as a difficulty in sustaining attention with increasing time-on-task. Previous research discussed in this chapter indicated the key role of right fronto-parietal regions in maintaining attention over time. Considering that non-lateralised attention deficits tend to persist, I then explain that the ultimate aim of this thesis is to explore the potential of a treatment involving the application of non-invasive brain stimulation to remediate vigilant attention deficits in patients with right hemisphere damage.

Based on these premises, in the second Chapter I describe my methodology for identifying the affected population. I present the methods used for screening individuals on acute stroke wards to identify patients with attentional impairments. These are the individuals who, in the chronic stage of their illness, were invited to take part in the studies described in this thesis. The expectation was that, in more chronic stages, lateralised deficits would have resolved or improved, whereas vigilant attention deficits would still be persistent in the

majority of this population. Previous research has indeed shown that vigilant attention difficulties following right-hemispheric stroke are particularly severe and long-lasting, hindering the potential to benefit from neurorehabilitation (Bowen, McKenna, & Tallis, 1999; Denes, Semenza, Stoppa, & Lis, 1982). Considering that vigilant attention difficulties strongly contribute to post-stroke disability, and have been shown to be strongly associated with the chronicity and functional disability of neglect (Duncan et al., 1999; Hjaltason, Tegner, Tham, Levander, & Ericson, 1996; Husain, Shapiro, Martin, & Kennard, 1997; Robertson, Manly, Andrade, Baddeley, & Yiend, 1997), vigilant attention deficits may represent a potential rehabilitation target that so far has been underexplored (Olgiati & Malhotra, 2020; Van Vleet & DeGutis, 2013).

I then describe the development of a novel approach to modulate brain activity of a key area for vigilant attention, the right dorsolateral prefrontal cortex (DLPFC). Stimulation montage and modelling, including how tDCS is set up for intrascanner delivery, is detailed in Chapter III. Conventional tDCS utilises two large sponge electrodes positioned far apart, which results in the current travelling long distances in the brain and potentially influencing several collateral cortical and subcortical regions (Moreno-Duarte et al., 2014). The high-definition tDCS approach I use in this work, on the other hand, aims at increasing precision and optimal targeting of a region of interest by constraining the current to the area between the electrodes (e.g., see Datta et al., 2009). This can be achieved using denser electrode arrays, leading to substantial shunting of the current through superficial cortical layers without reaching deeper regions (Huang & Parra, 2019). Such approach could be especially indicated in individuals affected by focal brain injury, as it avoids current dissipation into the cerebrospinal liquid occupying the space once occupied by the lesion.

In Chapter IV, I present a double-blind sham-controlled crossover design study that sets out to explore the behavioural impact of targeted tDCS in healthy individuals across the life span. In this study, I applied targeted tDCS during task performance in a sample of younger and older healthy individuals. I found that

tDCS is able to improve target detection (i.e., omissions and accuracy) in a cognitive paradigm that requires participants to maintain concentration over 15 minutes. The effect on accuracy was more pronounced in the older group, as compared to younger healthy adults. In addition, I found that a working memory measure is susceptible to the application of offline tDCS, with brain stimulation affecting false alarm rates on the n-back task.

I then moved on to apply the experimental protocol to stroke patients. In Chapter V, I outline the attention-modulating effect of electrical brain stimulation targeted to the right prefrontal cortex in a sample of right-hemispheric stroke patients. I show that the targeted delivery of brain stimulation to an intact cortical region is efficacious in reducing the total number of missed targets on the vigilance task. These results further support the causal role of the right DLPFC in attentional performance, and highlight the potential of this approach in ameliorating post-stroke deficits.

Finally, anatomical correlates of performance and response to stimulation are the subject of the investigation presented in Chapter VI. Performance on the vigilance task is found to depend on lesion location, rather than general lesion volume. The importance of two large white matter tracts, the right corpus callosum and the right cingulum, emerged, suggesting a potential role of inter-hemispheric and fronto-parietal disconnections in vigilant attention dysfunction. By using lesion anatomy methodology, I also show that the subset of patients who did not respond to the application of tDCS had brain damage centred on the right thalamus and postcentral gyrus.

A novel combined tDCS-fMRI study assessing the inherent functional organisation of the brain during stimulation is also described in Chapter VI. The application of tDCS was found to be capable of increasing functional connectivity within the left executive control network (LECN) across groups, i.e., healthy individuals across the life span and stroke patients. This finding emerged consistently, using different analysis approaches, corroborating its robustness. This suggests that the network-

modulatory effect of targeted tDCS is not targeted; instead, tDCS may modulate large-scale distributed brain networks, influencing connectivity in the hemisphere contralateral to the stimulation.

By adding the information about network response to tDCS into the equation, it was also possible to interrogate whether the scale of the changes in network connectivity during stimulation predict behavioural response to tDCS. I show that tDCS-induced increased connectivity within the right executive control network and reduced connectivity within the default mode network are associated with greater behavioural response to tDCS in the patient group.

2 Strengths of my approach

2.1 Study design

The behavioural and imaging studies described in this thesis were sham-controlled. This means that tDCS was always compared against a control condition, where the stimulation was delivered for a few seconds to mimic the sensations of real tDCS. In a within-subject crossover design, all participants were randomised to the active treatment and the placebo sham treatment. Individuals would receive both treatments in randomised order, therefore serving as their own control. This technique, known as counterbalancing, is typically assumed to deal with order effects in repeated measures designs. Considering that the administration of stimulation may have a carryover effect though, I also controlled for an effect of giving two slightly different treatments to the two groups by including the factor 'stimulation order' (i.e., the chronological order of stimulation) as a co-variate in my analysis (e.g., as in Labree, Corrie, Karolis, Didino, & Cappelletti, 2020).

2.2 Sample

I am aware that study findings can be generalised if the selection process is well designed and the sample is representative of the general population. The number of participants for my main study, looking at the attention-modulating effect of tDCS in patients, was arrived at following a power calculation.

Importantly, in addition to having sufficient participants, I invested time and allocated resources from my PhD research grant to recruit a truly representative sample. This included relatively disabled individuals, wheelchair bound, who are often excluded from investigations such as imaging studies. I believe that this approach allowed this body of work to be more inclusive, and its findings more easily applied to the wider population of stroke survivors.

Also, by using a theoretically driven approach focused on prefrontal brain areas involved in vigilance, I deliberately decided to target a core deficit that is likely to persist and strongly impact activities of daily living. My choice was also motivated by the following considerations: i) the prefrontal cortex is typically spared in this patient population; ii) many studies looking at ways to modify the syndromic features of neglect encountered difficulties in demonstrating a group effect in robust designs, but non-lateralised attention deficits have been underexplored.

2.3 Optimal stimulation protocol

The tDCS protocol used in this body of work was specifically optimised for delivery in a patient population. The choice of a montage that would enhance focality was motivated by the desire to constrain the current in a spared region of the brain, minimising potential current dissipation. By targeting survival cortex involved in the ability to maintain attention, I aimed to influence activity in large-scale attention networks typically damaged in neglect.

Importantly, the tDCS montage that was developed was well tolerated and acceptable to healthy participants and patients alike. Reported side effects were very mild, in all cases.

2.4 Lesion anatomy

The vast majority of patients tested in this thesis were scanned using high-resolution MRI sequences, and lesions were traced onto their native scan, using other modalities to assist with map delineation. This method represents the gold

standard of lesion mapping, allowing to draw better comparisons between behaviour and brain damage in specific areas.

2.5 Multimodal approach

A recent key methodological advance toward helping understanding the neural mechanisms of tDCS effects involves the integration of tDCS with concurrent techniques (Knotkova, Nitsche, Bikson, & Woods, 2019). TDCS integration with resting state MRI allows the experimental manipulation of brain activity and examination of associated changes in network connectivity. It is only in the last decade that this approach has increased in popularity, with the majority of existing tDCS-fMRI studies investigating the mechanistic underpinnings of tDCS delivered with conventional electrode placement (i.e., typically two 5x7cm electrodes) in young healthy individuals. In the imaging study I performed, outlined in Chapter VI, concomitant imaging was used during the delivery of high definition brain stimulation to characterise the effect of the targeted delivery of tDCS on brain networks, in healthy and clinical populations.

In sum, the robust experimental design used in this work put me in a strong position to establish whether there was any genuine effect on behaviour attributable to the intervention.

3 Limitations

3.1 Feasibility

An inherent limitation of tDCS at the present moment is the proportion of individuals who are screened out from studies because they do not meet the tDCS inclusion and exclusion criteria. This is particularly critical in clinical populations, who are in desperate need of a treatment to alleviate cognitive difficulties. In the studies described in this thesis, a significant proportion of patients were indeed screened out. Having had a seizure, which is not uncommon after stroke, represented the most common reason for exclusion. Some patients were also excluded as a precaution because they had undergone a cranioplasty; since the

distributions and effects of electric fields on surgically repaired skulls is less known (and would potentially require careful individual modelling), their inclusion would have constituted a potential safety risk.
The inclusion and exclusion criteria that are currently used in tDCS research to screen for potential candidates were initially derived from the TMS literature. They may now need to be reviewed to ensure that patients who could potentially receive the treatment safely are not denied participation (also see Bolognini et al., 2020). For instance, I would review the evidence that tDCS, especially when applied over intact cortex, may be contraindicated in cases of seizures. Given these issues, however, it is likely that tDCS will continue to be indicated for some stroke patients, and not appropriate for others.

3.2 Choice of task

The effect of brain stimulation on task performance that was observed in this research was found on task omissions across groups. In healthy adults, the effect of stimulation on the vigilance task was also found to affect other outcome measures via an interaction with treatment order. In retrospect, a potential way to at least mitigate the practice effect encountered in healthy individuals would have been to use parallel versions of the vigilance task, randomising letters and their location on the screen across sessions.

The choice of this task was motivated by the demonstrated ability of a shorter version of the same paradigm to detect a vigilance decrement in the relevant clinical population (Malhotra, Coulthard, & Husain, 2009). Following on from this, the aim of the present study was that of examining whether performance on a longer version of this task was amenable to the targeted application of tDCS, in different populations. The experimental paradigm, adopted to test all participants, was able to detect a worsening of accuracy with increasing time-on-task in stroke patients but not in healthy adults. My findings show that on this task, designed to detect a deficit in a clinical population, healthy adults performed at high accuracy levels throughout the task. Perhaps a longer task would have been more suitable to

observe changes in vigilance over time in healthy populations. Having said that, by using a consistent experimental strategy across all groups, an improvement in overall attentional performance following the application of brain stimulation was demonstrated across all participants, which adds to the validity of the approach.

Another general concern is that different types of vigilance tasks may measure different aspects of attention. The change in performance over time has been tracked and manipulated using different experimental paradigms in the literature which, at the broadest level, require responses to frequent and non-frequent targets (e.g., see Davies & Parasuraman, 1982; Helton & Warm, 2008; Nuechterlein, Parasuraman, & Jiang, 1983). In this work, I used a paradigm requiring responses to rare targets which had been shown to be able to detect a vigilance decrement (i.e., a decline in performance with increasing time-on-task) in stroke patients. The task was chosen because the primary aim of the study was to examine the effect of stimulation on this phenomenon, which is by some researchers considered to be the key measure of vigilance. As participants were asked to withhold their response in most trials, a fine-grained analysis of time-on-task was not achievable with this task, as this analysis would need much higher rates of responses.

3.3 Online and Offline tDCS

The studies described in Chapters IV-V (behavioural) and Chapter VI (imaging) employed online and offline tDCS methodology, respectively. These may tap into closely related but not identical stimulation mechanisms. TDCS is considered to exert its effect on the networks that are most active, but it is unlikely that exactly the same configuration of brain networks that is most active during task is also most active during rest. This issue demands particular caution if the main aim of the work is predictive, i.e., examining whether network response can predict behavioural response to a potential treatment. The primary aim of the present study was however to establish the efficacy of a potential treatment, and not to predict response to tDCS from network response.

If prediction were the main aim, an identical (online or offline) approach should be utilised for the behavioural and imaging experiments. One possibility would have been to also use an offline paradigm in the behavioural study, with stimulation administered at rest and vigilant attention tested before and after receiving tDCS. However, considering that the aim of the study was to test the efficacy of a potential treatment for attentional difficulties that could be used in a rehabilitation setting in the future, online tDCS (i.e., stimulation coupled with a task or cognitive training) was considered to be a more appropriate approach, potentially leading to a greater enhancement in performance as compared to offline tDCS (Oldrati, Colombo, & Antonietti, 2018).

Alternatively, one could argue that task-fMRI, as compared to resting-state fMRI, could have been a sensible approach - both studies would have effectively been online studies. However, vigilance testing in the noisy scanner environment is far from ideal, and not directly comparable to testing in the quiet laboratory environment, with the banging noise of the acquisition potentially influencing arousal levels. Also, having patients perform a task in an MRI machine is difficult in this population, and it would have probably increased drop-out rates and exacerbated scanner tolerability. As I particularly valued inclusivity here, as stressed above, the use of resting-state fMRI methodology was preferred to task-fMRI, because this approach is particularly advantageous in clinical populations. By removing the burden of performing a task in the scanner, I made the study more inclusive and the findings more transferrable to the general stroke population. This means that the sample was less subject to the bias of over-representing mildly affected individuals, allowing me to draw conclusion on treatment efficacy across the whole sprectrum of severity.

It would have been certainly interesting to explore whether the intrascanner application of tDCS used in the imaging study (Chapter VI), which led to an online increase in connectivity within the left executive control network, would have also been accompanied by a behavioural change in performance on a task performed afterwards. In order to do this, I could have asked participants to perform the

vigilance task pre and post tDCS-fMRI, and examine the efficacy of a treatament involving the offline application of tDCS. This would have enabled me to establish whether the targeted delivery of tDCS is also efficacious when delivered in an offline design, which may represent the only viable option for patients who are not well enough to perform a computer task. It should be noted that the patients I recruited were all able to engage with the vigilance task used in this work. The possibility to add a re-test phase after imaging was considered in the design phase of the study. However, having patients with attentional deficits repeat an attentional task at the end of a long session was considered to be too taxing on our population of severly impaired stroke patients, and would have potentially increased drop-out rate. This information could have been potentially collected in healthy populations at least. A main limitation of this approach would be the lack of a control group, because all participants in the present study received brain stimulation whilst in the scanner. A valid comparison here would be having a control group of adults, in a parallel group design, performing the task twice, before and after sham tDCS. This control experiment could help disambiguate whether any observed effect was related to the appication of tDCS, or due to a practice effect.

3.4 Inter-individual variability

An important question in the tDCS literature relates to the typical impact on behaviour that can be expected with stimulation. At the group level, the experiments presented here showed that a single application of tDCS significantly improved target detection in healthy adults across the life span and, most importantly, in patients with attentional impairments. However, at the individual-level, patient response to treatment varied. Full understanding of individual characteristics is likely to be of greater importance in patient studies, but it is particularly difficult to achieve because this involves the additional variability in clinical presentation and stroke related factors (e.g., injury type, lesion volume and location, stage of recovery). In addition, our understanding of how these factors influence post-stroke recovery is limited.

The poorly comprehended inter-individual variability of response to tDCS has been one of the main obstacles to a clinical translation of tDCS, strongly limiting the establishment of the cognitive impact of tDCS and hindering the sensitivity of clinical trials.

3.5 Effect on other cognitive functions

Another open question relates to the impact of the tDCS approach used in this work on other cognitive skills, such as executive functions and lateralised visual attention. My preliminary findings in healthy adults across the lifespan suggested that 10 minutes of high definition prefrontal tDCS may enhance performance on a working memory task performed offline. Specifically, tDCS reduced false alarm rates on the n-back task. It is possible that, by modulating the ability to sustain goal-oriented behaviour, tDCS may have indirectly induced a reduction in impulsive responses. A number of studies have shown that vigilance is necessary for effective executive functions (e.g. see Ponsford & Kinsella, 1992). Behavioural vigilance training significantly improved executive functioning decline in older adults and patients with mild traumatic brain injury (Van Vleet, Chen, Vernon, Novakovic-Agopian, & D'Esposito, 2015; Van Vleet et al., 2016). Neuromodulation studies aiming to target executive functions also positioned the electrodes over the prefrontal cortices, with encouraging results (e.g., see Reinhart & Nguyen, 2019). However, in this study, the effect of tDCS on attention and working memory partially diverged, as online tDCS did not seem to affect false alarms on the vigilance task – the effect was on omissions. It is possible that such differential effects on different outcome measures are related to the stimulation protocol (e.g., online vs offline tDCS design). To help disambiguate this, online tDCS targeted to the right DLPFC could be applied concurrently to the n-back in future studies. In addition, the effect of tDCS seemed to be (at least partially) mediated by the thalamus, which is also involved in response inhibition (see Section 4.8). The reason why this manifested in a reduction in false alarms on the n-back but not on the vigilance task may be linked to an intrinsic difference between the two paradigms (e.g., higher false alarm rates on the n-back).

The presence of any effect of tDCS on lateralised bias was explored in the sample of patients, but not in the sample of healthy adults. This could have been carried out by having healthy adults perform a variant of the line bisection task such as the landmark task (Milner, Brechmann, & Pagliarini, 1992), and examining whether performance could be modulated by prefrontal tDCS. On these tests, older adults typically show pseudoneglect, a pattern of performance suggestive of a bias in favour of stimuli appearing in the left visual space (Bowers & Heilman, 1980). Pseudoneglect appears to be amenable to experimental manipulations (e.g., line length and time-on-task), which have been shown to reduce this phenomenon and produce a rightward shift in performance (Benwell, Harvey, Gardner, & Thut, 2013; Benwell, Harvey, & Thut, 2014). Whether targeted prefrontal tDCS would also be able to affect spatial bias in healthy adults could be addressed by future investigations. In patients, however, no effect of tDCS on the spatial bias was detected at the group level, using the pencil-and-paper version of the line bisection task. Furthermore, the tDCS approach used in this work did not seem to be able to improve other clinical measures of the lateralised bias of neglect in stroke patients, at least when assessed offline.

3.6 Lesion anatomy of tDCS response

The neuroanatomical findings presented here require careful consideration, as voxel-based lesion symptom mapping is not without caveats (for a review, see Malhotra & Russell, 2015). An important limitation stems from the making of a binary decision as to whether a tDCS response is present or not, and splitting patients into responders and non-responders on the basis of a change in performance. Clearly, it follows that different outcomes would be yielded dependent on the cut-off points utilised to categorise what was considered a response.

Also, this method does not take into consideration that brain functions seem to work in a distributed manner (Fox & Raichle, 2007). Lesion anatomy of neglect is mainly subcortical (Corbetta et al., 2015), leading to disconnections and

dysfunctions in the interplay between cortical regions. However, a preliminary analysis aimed at linking network and behavioural response to tDCS across groups was performed as part of this thesis and will be discussed in Section 4.9.

3.7 Power

Statistical power refers to the probability of concluding that there is an effect in a population when the effect indeed exists (Cohen, 1992). Power is usually derived from previous findings, using estimates of error rates and effect sizes. In the early stages of the research design, I attempted to ensure adequate power to detect an effect of tDCS on online task performance (the main aim of the present research) by performing a power calculation based on the estimated effect size.

My studies were however not powered to detect changes in offline measures such as working memory tasks in healthy adults and neglect tests in stroke patients. Following the application of brain stimulation, behavioural changes emerged in the working memory task in healthy adults, but not in clinical measure of visual search efficiency in patients. Further powered studies are needed to specifically address the potential of offline prefrontal tDCS to modulate working memory as well as lateralised deficits of neglect.

Power analysis is more complicated in fMRI studies than in behavioural ones. The imaging study described in Chapter VI was not preceded by a formal power calculation. Sample size (a total of 66 individuals) for my imaging study was kept i) comparable between groups (i.e., at least n=20 per group of younger and older healthy adults and stroke patients); ii) aligned with the sample of individuals recruited for my behavioural studies; iii) comparable if not greater than previous studies (e.g., see O'Shea et al., 2014). New tools are now available to assist researchers with calculating power in fMRI studies (e.g., http://neuropowertools.org), but a significant increase in sample size comes at high research costs of data collection. To increase power, in the imaging study described in Chapter VI, Section 4, a crossover design was preferred to a between-

subject design, with each participant undergoing two fMRI runs: real and sham tDCS. In addition, considering the huge number of comparisons between voxels that are carried out in imaging studies, I adjusted for multiple comparisons using the threshold-free cluster enhancement (TFCE) method (Nichols & Holmes, 2002), which does not consider each voxel as independent from its surrounding neighbours but uses the concept of 'clusters' of signal, providing better sensitivity in finding real sources of signal, as compared to voxelwise thresholding.

4 Future investigations

Along the whole experimental body of the thesis, two main components were consistently used: the targeted neurostimulation approach, specifically devised to constrain the delivery of brain stimulation, and the computerised paradigm measuring the ability to maintain attention to spatial location over time. This was a deliberate choice, motivated by the reasoning outlined in the next sections outlining future investigations following on from my original findings.§

4.1 tDCS research

tDCS is a neuromodulation method that consists of passing a weak electrical current across the cortex via electrodes positioned on the scalp. Throughout this thesis, I have avoided describing tDCS as having an "excitatory" or "inhibitory" impact on brain function, because this has been shown to be too simplistic in many cases; instead, I described the specific setup, focusing on the flow of the induced electrical field from the anode (the source) to the two cathodes (the sinks). By using a triangular montage clustered around a part of the attention network that is frequently spared by stroke, the risk of shunting of the current was kept to a minimum. The aim of this was to maximise the chance of detecting an effect of tDCS at the group level, as the current was in all cases delivered to surviving cortex.

As delivering stimulation to surviving cortex was considered key, patients that had lesions encroaching the target area were excluded from participation in the study.

Future studies directly addressing to what extent these individuals may be unable to benefit from a treatment involving tDCS delivered to damaged regions would be desirable. If a lack of an effect was confirmed, alternative montages targeting intact areas of the same network could be tried to explore other possible avenues.

It is important to note that the effects of tDCS that have been described in this work are dependent, at least to some extent, on the specific stimulation paradigm that has been used. Other possible extensions of this work would include explorations of the role of parameters such as the stimulation duration, intensity and polarity as well as the electrode configuration used. Previous studies suggest that the effect of tDCS may be non-linear, with for instance higher intensities or longer periods of stimulation not necessarily associated with an increase in efficacy, but potentially leading to a shift in the excitation/inhibition pattern (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Shilo & Lavidor, 2019).

Finally, in addition to the above-mentioned considerations, it would be important to include tests of reliability to confirm that the effects of any given tDCS configuration are stable and reproducible.

4.2 Vigilance task

Future investigation aiming to examine moment-to-moment fluctuations could consider using a task with frequent targets such as the SART (Robertson, Manly, Andrade, et al., 1997). Future studies could explore whether the findings presented here hold when using such paradigms – a replication with a different task would confirm that the effect is not task-specific, but process-specific.

As expected, the task was able to detect a vigilance decrement (in RT and target detection) in stroke patients. In healthy adults, a decrement in RT over time was demonstrated. Importantly, the vigilance decrement did not appear to be modulated by the application of tDCS in either population. This is in contrast with a previous study that indicated that tDCS can modulate the vigilance decrement in

target sensitivity in younger healthy adults performing a simulated air traffic controller task lasting for 40 minutes (Nelson, McKinley, Golob, Warm, & Parasuraman, 2014). Data from the majority of the now available evidence pooled together, including the present research, however indicates that tDCS does not counteract the vigilance decrement (e.g., Brosnan et al., 2018), with differences potentially to be found in the methods that have been used, especially the chosen task. Future studies could continue to provide evidence to this debate.

4.3 Online and offline tDCS

In a proof of principle study, I examined the efficacy of a potential treatment involving tDCS combined with task performance. Concurrent tDCS-fMRI also provided useful information on network response during tDCS. These designs were considered optimal for the aims of this thesis. More studies looking at the direct comparability of the effects of online and offline tDCS are needed (Olgiati & Malhotra, 2020).

4.4 Effect on other cognitive functions

The behavioural studies described in Chapter IV and V were not powered to fully detect the potential of the proposed approach to influence working memory and the lateralised attention component of neglect (see Section 3.7 'Power'). More research is needed to confirm and extend my initial findings.

Future studies may also evaluate the impact of targeting attention on other functions, such as motor abilities. Motor deficits and hemiparesis following stroke may indeed arise from attentional difficulties. Profound neglect may mimic and even exacerbate an underlying primary deficit (see review by Halligan & Marshall, 2002). The presence of concomitant attentional difficulties may account for the paradoxical higher incidence of primary sensorimotor deficits such as hemiplegia following right- than after left-hemispheric stroke (Sterzi et al., 1993). This opens up the possibility that by therapeutically targeting a domain-general function such

as attention by tDCS, potentially coupled with training, motor impairments may also improve (Rinne et al., 2018).

4.5 Inter-individual variability

In cases of heterogenous syndromes like neglect, it has become increasingly apparent that it is unlikely that a single treatment will work for every patient. It would be critical to determine which individuals will benefit from the application of each set of parameters of brain stimulation (responders). Retrospective analysis of treatment response like the one used in the present study can help identify subgroups of patients that benefit from a particular therapy. For instance, my study suggested that patients with lesions involving the thalamus and postcentral gyrus did not benefit from the intervention. Interrogation of lesion anatomy therefore revealed a key role of such anatomical loci in tDCS response.

Neuroimaging studies may also play a valuable role in uncovering the variables underlying response to tDCS. In a concurrent tDCS-fMRI study, I found a systematic increase in connectivity within large-scale networks across groups, particularly the LECN, during brain stimulation as compared to sham. My results add to the emerging body of work indicating that the whole-brain, large-scale network-level impact of stimulation is key, as opposed to the local impact of tDCS. I argue that multi-modal paradigms, with concurrent imaging carried out during stimulation, are particularly welcome in an effort to help with understanding the mechanisms behind the effects of tDCS, and shed light into the above-mentioned variability in response and the inconsistent results in the literature. A possible extension for future studies here would be to stratify patients into groups with similar functional and/or structural connectivity, and explore treatment effects in each subgroup (e.g., see Li et al., 2019). Evaluation of white matter tracts (which was acquired as ancillary as part of the protocol) could allow characterising response to tDCS. For instance, future analysis could investigate whether the integrity of the superior longitudinal fasciculus linking frontal and parietal areas (and in particular branches II and III, which originate from distinct parts of the inferior parietal

lobule, a key region for neglect), played a role in influencing the effect of targeted brain stimulation (Barbeau, Descoteaux, & Petrides, 2020).

In sum, the identification of predictors of behavioural response to tDCS in stroke patients would strongly favour a clinical translation of tDCS and should be encouraged. Predicting the response to neuromodulation in person-centred approaches in clinical practice, tailored to the individual, represent a key challenge for precision modulation. When clinicians see a patient, they are confronted with the challenge of predicting outcome of each available treatment and/or combinations of treatments. A classifier, an algorithm that maps data to categories, may help predicting cognitive outcome at the individual level in a clinical setting, providing percentages of success of each treatment or combination of treatment given certain risk factors (e.g., lesion location). Multivariate models could then be updated as more data and longitudinal datasets become available. This would also help Clinical Psychologists supporting patients and their families having more realistic expectations of outcome.

Results from this proof of principle study are encouraging, and replications in this clinical population would be important to further this research. A high quality randomised clinical trial is justified to compare a range of treatment healthcare interventions (including tDCS combined with a behavioural intervention) for attentional impairments following right-hemispheric stroke. The intercollegiate Stroke Working Party of the Royal College of Physicians indeed recommends offering patients interventions ideally in the context of a clinical trial (Bowen, James, & Young, 2016). Phase III-IV efficacy trials would have to be preceded by a feasibility study, which will also inform power calculations for sample sizes. Considering the well-known patient heterogeneity, and the likelihood that patients will respond to different treatments, the cohort is anticipated to be large, most likely addressed in a multi-centre study. Such feasibility study would also provide valuable information on potential barriers of performing, for instance, a study across the country delivering treatments in inpatient settings, outpatient neglect clinics and home set-ups. This strategy would support the evaluation of the

efficacy of different treatments for right-hemispheric stroke patients and would help build models that can predict outcome and response to different treatments.

4.6 tDCS as a rehabilitation tool

In a proof of principle study presented in Chapter V, I have shown that performance on an attentional task can be modulated by one session of tDCS in a sample of chronic stroke patients.

At present, there is scarce evidence to suggest an optimal therapeutic window for tDCS administration. A number of studies have failed to demonstrated a value of tDCS is in acute stages post-stroke (Di Lazzaro et al., 2014; Rossi, Sallustio, Di Legge, Stanzione, & Koch, 2013). Most studies in the literature have applied tDCS in the post-acute and chronic post-stroke phases, allowing a degree of spontaneous recovery to occur and potentially peak before applying tDCS.

This also means that tDCS can be specifically used to target persistent deficits which are unlikely to recover and likely to affect activities of daily living. In relation to the neglect syndrome, top-down deficits in the ability to sustain attention, as compared to lateralised deficits, may better account for chronic difficulties experienced by patients. It has been suggested that the spatial bias in neglect recovers faster than the general (vigilant) attention deficits (Karnath, 1988). Indeed, the spatial bias seems to improve most rapidly in the first 2-3 weeks post stroke (Hier, Mondlock, & Caplan, 1983; Stone, Patel, Greenwood, & Halligan, 1992; Wade, Wood, & Hewer, 1988), whereas vigilant attention deficits tend to persist and have been found to be associated with poorer functional outcomes following stroke (Duncan et al., 1999; Hjaltason et al., 1996; Husain et al., 1997; Peers, Cusack, & Duncan, 2006; Robertson, Manly, Beschin, et al., 1997). In a recent study by Nurmi and colleagues (Nurmi et al., 2018), 65 right-hemispheric stroke patients were followed up longitudinally in their first year after stroke and at re-test, there was a reduction in the spatial bias as measured by two cancellation tasks. Omissions were more evenly distributed in the space – a

pattern which more suggestive of a persisting problem with vigilant attention than ongoing pathological bias. Despite the significant dropout rate recorded for this study, and the fact that the clinical sample did not include any cases with the full-blown syndrome, the study highlighted how individuals with milder neglect may have persisting problems with their general attention that are typically under-recognized and under-treated in clinical work. This trajectory has also been reflected in my own clinical experience, having worked closely for over 10 years with patients with neglect.

In a future trial, the efficacy of a treatment involving multiple applications of tDCS on a clinical endpoint, not just a cognitive measure, would be appropriate. Studies exploring the efficacy of this approach in the context of a rehabilitation protocol must include outcome measures such as the effect on functional tasks and activities of daily living like dressing and mobility, as recommended by the intercollegiate working party of the Royal College of Physicians (Bowen et al., 2016). Other meaningful information would include discharge destination, quality of life and disability measures.

Rehabilitation studies may use tDCS paired with behaviourally-based clinical interventions to further augment its effects. For instance, a study showed that pairing brain stimulation with cognitive training produced, as compared to training only group, the greatest and longer lasting enhancement in numerosity discrimination, and more importantly, such improvement was transferrable to untrained related tasks (Cappelletti et al., 2013). Even if more research is needed to confirm the incremental value of coupling of tDCS with training in a rehabilitation setting, a recent meta-analysis of studies found encouraging moderate-quality evidence of the effectiveness of non-invasive brain stimulation (including but not exclusively tDCS) combined with behavioural treatments (Salazar et al., 2018).

Drug-augmented stimulation could also be explored in future investigations examining the combination of pharmacological and electrical stimulation. For

instance, the efficacy of the combined administration of tDCS and noradrenergic (Dalmaijer et al., 2018) or dopaminergic (Gorgoraptis et al., 2012) medications could be explored, ideally in a factorial design to test the drug and stimulation interventions individually and simultaneously (i.e., sham tDCS, real tDCS, pharmacotherapy, combined therapy).

Because of the device configuration and portability, tDCS could be set up for use in the home environment, ideally supervised in virtual clinic settings, which ensures adherence to guidelines and procedures for safe and reliable tDCS. The idea of a home-based tDCS treatment is very attractive: it may decrease costs, favour inclusion of patients who may not be able to travel to the city daily to receive treatment, and open up new possibilities for helping seriously ill patients, for whom daily travel to a treatment facility is burdensome or just not possible (Knotkova et al., 2019). It is impractical to think that stroke survivors will be able to set this up without direct assistance of a family member, most often due to motor weakness of one side of the body and the problems which are the subject of the intervention, attentional impairments, which would obstruct engagement in the activity. Even in home-based therapy setting though, it is likely that only the most motivated individuals/family will complete a cycle of treatment. Specific recommendations for best tDCS home-delivery tailored to this group of patients are yet to be developed.

4.7 Trial feasibility

In relation to the previous point (i.e., the potential use of tDCS as a rehabilitation tool), it is important to note that feasibility of clinical trials involving the use of tDCS in the context of neglect rehabilitation has been recently indicated as a critical issue. A recent investigation mainly attributed this to the difficulty in travelling to a research centre every day to receive a treatment, which can be particularly daunting for a population that is normally discharged home following the initial period of assessment (Learmonth et al., 2020). Considering that many patients would be at home in a chronic post-stroke stage when trials normally take

place, transport would involve notable costs (for families, or costed in a research grant), especially if there is motor weakness which requires the use of a wheelchair. To broaden the sample and give the opportunity to benefit from a potential treatment to as many patients as possible, future clinical trials could include cases whereby tDCS is delivered in patients' homes, ideally in remote clinic virtual settings. Given the lack of treatment options for this patient group, and the documented impact of attentional difficulties on everyday life, I believe that future efficacy trials are justified, but of course feasibility and repeatability would need to be evaluated in addition to this. Given the known difficulty to recruit patients for such studies, it would be appropriate to review tDCS inclusion and inclusion criteria, which were initially derived from TMS studies but could be adjourned with evidence accumulating (also see Bolognini et al., 2020; Learmonth et al., 2020). Moreover, as the technology for the delivery of tDCS improves, the potential for administering tDCS more routinely will increase. Finally, I was able to deliver tDCS in the scanner to over 20 patients. This required considerable effort and logistical planning, greater than that required for any efficacy study.

4.8 Lesion anatomy of tDCS-response

The lesion subtraction analysis was used to identify regions that are critical for a response to tDCS to occur. Potential neural correlates of tDCS responsiveness were the right thalamus and the right postcentral gyrus.

The largest subcortical output of the prefrontal cortex (the stimulation target in my study) is the thalamus, which is a relay station for motor and sensory information, with bilateral connections from and to the cortex. The cortical-striatal-thalamic-cortical circuit, which connects the prefrontal cortex, the basal ganglia, and the thalamus in one continuous loop, is considered to be one of the main structures responsible for response inhibition and reward processing, which modulate attention (Graybiel, Aosaki, Flaherty, & Kimura, 1994; Olgiati, Russell, Soto, & Malhotra, 2016). Also, in a neat set of experiments in patients, de Bourbon-Teles and colleagues showed a pivotal role of the thalamus in linking visual

memory and attention: thalamic patients were found unable to use a visual cue for navigating an attentional task (de Bourbon-Teles et al., 2014). In a recent meta-analysis of fMRI studies, the thalamus was identified as one of the clusters that consistently showed activation during vigilance tasks (Langner & Eickhoff, 2013). Portas and colleagues found increased attention-related activity in the thalamus in conditions of low arousal such as in sleep deprivation, and concluded that the role of the thalamus would be that of mediating the interaction between arousal and attention (Portas et al., 1998). Recently, a diminished network engagement in fronto-parietal-subcortical circuits, including the thalamus and subthalamic nucleus, was showed in individuals with cocaine addiction with attentional disfunction, as measured by a vigilance task such as the stop signal task (Wang et al., 2018). Lesions of the thalamus are indeed known to produce neglect manifestations in humans (Karnath, Himmelbach, & Rorden, 2002; Singh-Curry, Malhotra, Farmer, & Husain, 2011). In a single-case design, Singh-Curry and colleagues reported the case of a gentleman with persistent hemiparesis and severe arousal and attentional deficits, including lateralised and non-lateralised vigilant attention deficits, secondary to acute disseminated encephalomyelitis (ADEM) (Singh-Curry et al., 2011). MR imaging revealed bilateral thalamic involvement, with additional small lesions outside neglect-producing areas, such as the temporal and occipital lobes and the cerebellum. This patient responded very well to the administration of the noradrenergic agonist guanfacine, leading to an increase in NA availability which is produced in the locus coeruleus which then projects to the thalamus and to the frontal and parietal cortices. It is therefore possible that, in my work, thalamic damage and/or disconnection may have prevented tDCS having a widespread effect on attention networks, as the thalamus would have been functionally disconnected from either the stimulated area (the right DLPFC) or other key regions for attention such as the posterior cingulate. Importantly, a role for the thalamus also emerged from my network analysis. In the patient group, the right thalamus was found to respond with an increase in connectivity to regions within the left executive control network during real tDCS, as compared to sham tDCS. A potential way to put the role of the thalamus to test would be to selectively recruit a group of patients with thalamic damage and

directly compare their response to a treatment with tDCS with that of a stroke control group without thalamic involvement. Functional connectivity of the thalamus could also be specifically examined in future studies, as will be discussed in Section 4.9.

The right postcentral gyrus was also found to be involved in response to tDCS. This gyrus, posterior to the central sulcus, is located in the lateral parietal lobe and contains the primary somatosensory cortex. More recently, its proprioceptive gaze input has been discovered, hence a possible role of this area in the ability to code the locus of attention (Wang, Zhang, Cohen, & Goldberg, 2007). The task used in this study has a spatial component, and tDCS may have exerted its modulatory effect by boosting this aspect of task performance. Fixation recording or comparison of spatial with non-spatial vigilance tasks could help future studies to disambiguate this.

One interesting extension of this work examining anatomy of response would be to examine for frontoparietal disconnections in this patient population. Specifically, I would be interested to link volume of the superior longitudinal fasciculus (connecting frontal areas with parietal lobe and temporoparietal junction) and response to tDCS. DTI was acquired in all participants, although analysis of this dataset was not part of the current thesis.

4.9 Networks of tDCS response

Findings from the multimodal imaging study of brain network synchronisation during real and sham tDCS across healthy and clinical populations are discussed below.

Local connectivity. On average, patients showed preserved functional connectivity for the stimulation target, i.e., the right dorsolateral prefrontal cortex. This is unsurprising, as they had been selected to take part in the study because this area was intact in a previous inspection of imaging. Such local connectivity was not

modulated by the application of tDCS, and mean networks strength extracted for the area encompassing the electrodes was comparable during real and sham tDCS. This finding suggests that the modulatory effect of the targeted application of tDCS is not focal but rather more widespread, transmitting from the stimulation site to remote but connected areas. This is in keeping with other studies that, using traditional tDCS montages, showed that the modulatory effects of tDCS are not limited to the area underneath the electrodes, but rather affect distant regions (e.g. see Cabral-Calderin et al., 2016; Li et al., 2019). In a recent study, EEG was acquired concurrently to brain stimulation as older adults were performing a vigilance task (Brosnan et al., 2018). During stimulation, which was implemented with two large sponge electrodes in a F4-CZ tDCS montage, researchers showed enhanced EEG markers of early visual evoked responses in occipito-parietal regions as well as signs of frontal engagement, i.e., strengthened amplitude of the P2 component, distributed around centro-frontal areas on the scalp. Even if it was not possible to establish from this study whether there was a different modulation in the left and right frontal areas, because researchers averaged neighbour electrodes from the two hemispheres to obtain the frontal component, the modulatory effects of tDCS appeared to be widespread and not confined to electrode locations. A direct comparison between this study and my approach is prevented by the methodological differences. However, my findings in over 60 participants extends those of previous studies by showing that widespread changes in connectivity may be expected when using targeted montages too.

Large-scale connectivity. During tDCS, an increase in functional connectivity within the left executive control network (LECN) emerged, as compared to sham tDCS. TDCS affected within network connectivity contralaterally to the stimulation site (and contralesionally for patients) across all groups, with no significant differences between groups. Within this network, different clusters of increased mean connectivity were identified in both hemispheres during real as compared to sham tDCS. In healthy adults, clusters of increased connectivity within the LECN were detected in the right prefrontal cortex and left parieto-occipital cortex; in stroke patients, in the right thalamus and the left corona radiata.

This finding was confirmed by two further analyses focusing on intrinsic brain network connectivity. Mean connectivity strength for each network of interest was extracted and contrasted using two different masks, i.e., using the group mean average as a mask vs an independent mask derived from an anatomical atlas. Both methods highlighted a significant increase in connectivity strength within the LECN during tDCS, as compared to sham, across all groups.

The increase in connectivity in the hemisphere contralateral to the stimulation is a robust, consistent and intriguing finding. This is the first study to show an online modulation of a contralateral network induced by targeted tDCS across different groups. Previous studies have identified diffuse changes in connectivity following conventional tDCS to the prefrontal cortex in healthy individuals (e.g., see Keeser et al., 2011; Sankarasubramanian et al., 2017). Interestingly, in the study by Sankarasubramanian and colleagues, anodal stimulation of the right DLPFC was found to modulate thalamocortical networks bilaterally. This is in keeping with involvement of the thalamus in tDCS responsiveness as shown by my lesion mapping analysis. This suggests that the thalamus could be the gateway of the effect in the contralateral hemisphere, as the two thalamic bodies are connected by the adhesio interthalamica running through the third ventricle. To specifically test for a key role of the thalamus, a seed-based correlation-analysis could be performed placing an anatomical seed in this region of interest and then characterising the connectivity pattern of this area.

Alternatively, the effect could be the result of an interaction between networks. In this study, I focused on examining the effect of tDCS within each network of interest; future studies could consider exploring tDCS-induced modulations in connectivity across multiple networks.

Behaviour:connectivity. A collateral analysis was carried out to correlate behavioural and network response to the application of tDCS. In the patient group, changes in network response (increased FC within the LECN during tDCS) were found to be related to behavioural response (increased task accuracy during real

as compared to sham tDCS). In particular, behavioural response to tDCS was correlated with a reduced connectivity of the default mode network and an increased connectivity within the right executive network in response to tDCS. Tasks activating the executive network have been consistently shown to induce deactivation in the default mode network (Fransson, 2006). In this study, however, participants were at rest, and were asked to keep their eyes closed without falling asleep. In the absence of a task, the degree of tDCS-induced decrease in FC within the default mode network, the task negative network, and an engaged right executive network (which includes the stimulated DPFC), predicted a positive response to this treatment. This finding has to be interpreted with particular caution, considering that it stems from the combination of a study that measured behaviour during tDCS and a study that measured resting networks during tDCS (i.e., online and offline study) - the networks most active during each study, upon which tDCS exerted its effects, may have not necessarily be the same.

Between groups differences. Critically, no systematic difference in network response to the delivery of targeted prefrontal tDCS emerged between groups (healthy younger and older adults, stroke patients). The analysis of mean network strength revealed differences in mean connectivity that were unrelated to the application of tDCS. In general, patient mean connectivity within the salience, visuospatial and default mode networks was lower than that of healthy younger adults. These were networks that most likely were directly damaged by the right-hemispheric stroke. In contrast, the two executive control networks showed similar connectivity values – the left hemisphere was intact, and the integrity of the right prefrontal cortex was a prerequisite to study participation.

4.10 Blinding integrity

In the present study, participants were asked whether they thought they received brain stimulation, and to rate the intensity of any side-effects, after receiving real/sham tDCS combined with cognitive task or concurrently to brain scan. Brain

stimulation was safe and very well tolerated, with unremarkable contraindications reported.
Most (mild) side-effects were reported by younger adults, whose perception of stimulation was also above chance level, such that they could correctly identify stimulation condition. Previous studies also found that older participants experienced less discomfort during stimulation, as compared to younger adults; however, such studies did not report much information on participants' blinding (Gandiga, Hummel, & Cohen, 2006; Kessler, Turkeltaub, Benson, & Hamilton, 2012).

In the current work, patients were at chance level when asked to state whether they thought they received stimulation. Thus, in this heterogenous group, perception of stimulation was not mirrored by response to tDCS.

Future studies should continue to rigorously test and then report key information about blinding efficacy, e.g., what questions are used and at what time point sham-blinding is probed. For instance, a questionnaire could be administered during tDCS, immediately after stimulation or retrospectively at the end of the session, and this may steer participants awareness more towards the task or the stimulation, potentially influencing judgement.

While I was performing the studies described in this thesis, new data showing that high definition tDCS is associated with increased scalp sensations were published (Garnett & den Ouden, 2015). Increased skin sensation may place participants in a better position to tell real from sham, potentially because participants may be able to feel the sensations throughout the stimulation or for longer periods of time, and not just for a few seconds (Richardson et al., 2014). This highlights the need to find a better sham for HD-tDCS, especially when the target population is healthy younger adults, who may be more sensitive to the cutaneous sensations. One potential solution would be to use active stimulation of a control unrelated area (e.g., occipital). Alternatively, other researchers proposed to use a different tDCS configuration as a control whilst keeping the electrodes over the target area, e.g.,

comparing a ring configuration with a pair of adjacent electrodes strongly limiting the flow of the current (Garnett & den Ouden, 2015). Future studies should continue exploring alternative sham conditions whilst comparing the resulting behavioural effects. Having said that, these concerns seem to be less serious in older (Reckow et al., 2018) and stroke populations (present data), who find it harder to discriminate between stimulation and no-stimulation.

5 Final remarks

Neglect after right-hemispheric stroke is common and disabling. Given the well-known patient heterogeneity in presentation, it seems very unlikely that the one-size fits all approach will ever work to cure neglect. The road to recovery will probably have to encompass multiple interventions, offered as standalone treatment or in various combinations.

The key challenge in research is to augment the strength and duration of each treatment, and then find suitable combination of therapeutic axes (Harvey & Kerkhoff, 2015). Studies aiming to demonstrate potential of any intervention should test robustly, e.g., with adequate power, using multimodal techniques and increasing efforts to characterise the clinical population. Brain stimulation studies would also greatly benefit from modelling of current distribution, which is of particular relevance in populations affected by brain pathology.

This thesis sets out an investigation of a theoretically driven treatment involving the application of tDCS to alleviate vigilant attention deficits, a pervasive and persisting complaint among right-hemispheric stroke patients. The studies described in this thesis demonstrated the efficacy of the targeted electrical stimulation of the right prefrontal cortex in improving attention and modulating brain networks in different populations.

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APPENDIX I

Literature on tDCS for neglect

Table 1 summarises lesion studies using tDCS to improve lateralised bias in neglect.
Participants: Right-Brain Damage (RBD), Left-Brain Damage (LBD); Desired target area: Posterior Parietal Cortex (PPC), Parietal (P), Primary Motor cortex (M1); Outcome measures: Test of Attentional Performance (TAP), NEglect-Test (NET), Activities of Daily Living (ADL), Behavioural Inattention Test (BIT), Gamma aminobutyric acid (GABA), Barthel Index (BI), Catherine Bergego Scale (CBS), Clock Drawing Test (CDT), Functional Independence Measure (FIM).

Table 2 summarises lesion studies using tDCS to improve non-lateralised bias in neglect.
Participants: Right-Brain Damage (RBD), Left-Brain Damage (LBD); Desired target area: Dorsolateral Prefrontal Cortex (DLPFC); Outcome measures: Working Memory (WM).

TABLE 1

Study	Participants	Study design	Time post-stroke	Intensity	Duration	Sessions	Online/Offline	Coupling	Desired target	Outcome measures
Ko et al., 2008	15 RBD with neglect	Double-blind, sham-controlled, crossover	1-3 months	2 mA	20 min	1 real 1 sham	Offline	Rest	Right PPC	Line bisection, symbol cancellation, letter cancellation
Sparing et al., 2009	10 RBD with neglect (+ young controls)	Double-blind, sham-controlled, crossover	2.9±3.5months	1 mA	10 min	1 anodal 1 cathodal 1 sham	Offline	Rest	Right PPC	Computerised line bisection, visual search task
Sunwoo et al., 2013	10 RBD with neglect	Double-blind, sham-controlled, crossover	27.8±60.4 months	1 mA	20 min	1 real 1 sham	Offline	Rest	P cortices	Line bisection, star cancellation
Brem et al., 2014	1 RBD with neglect and hemianopia	Single-case	23 days	1 mA	20 min	1 daily per 5 days per 4 weeks	Online	Eye movement protocol	Right PPC	TAP, NET (German equivalent of BIT), ADL
O Shea et al., 2017	3 RBD with neglect (+ young controls)	Longitudinal case-series	Chronic	1 mA	20 min	1 real one sham	Online	10°prisms	Left M1	BIT, neglect battery (cancellation tasks and eye movements), GABA concentration (spectroscopy)
Smit et al., 2015	5 RBD with neglect	Double-blind, sham-controlled, crossover	58±52.27 months	2 mA	20 min	1 daily for 5 days	Offline	Rest	Right PPC	BIT total score
Ladavas et al., 2015	30 RBD with neglect (10 per group)	Double-blind, sham-controlled, between	3.2 months	2 mA	20 min	1 daily for 10 days	Online	10°prisms	P cortices	BIT total score
Bang & Bong, 2015	12 RBD with neglect (6 per group)	Double-blind, sham-controlled, between	1.5±1.5 months	1 mA	20 min	1 daily for 15 days	Offline	Feedback training	Right PPC	Line bisection, motor-free visual perception test, BI
Yi et al., 2016	30 RBD with neglect (10 per group)	Double-blind, sham-controlled, between	Subacute	2 mA	30 min	1 daily per 15 days	Online	Conventional OT	Right PPC	Line bisection, star cancellation, BI, motor-free visual perception test, CBS
Turgut et al., 2016	20 RBD, 12 LBD with neglect (16 per group)	Double-blind, sham-controlled, between	22.5±16 days	1.5-2 mA	20 min	1 daily for 8 days	Online	Optokinetic training	P cortices	Line bisection, apple cancellation, CDT, spontaneous body orientation, FIM, early rehabilitation/BI

TABLE 2

Study	Participants	Study design	Time post-stroke	Intensity	Duration	Sessions	Online/Offline	Coupling	Desired target	Outcome measures
Jo et al., 2009	10 RBD	Single-blind, sham-controlled, crossover	2.4±1 months	2 mA	30 min	1 real 1 sham	Online	Task at minute 25	Left DLPFC	Verbal WM: 2-back task
Kang et al., 2009	10 LBD/RBD (+ older controls)	Double-blind, sham-controlled, crossover	18±13 months	2 mA	20 min	1 real 1 sham	Offline	Rest	Left DLPFC	Go-No go task (30 trials)
Park et al., 2013	11 LBD/RBD (6 vs 5 patients)	Double-blind, sham-controlled, between	Unknown	2 mA	30 min	1 a day, 5 per week	Online	Computer-assisted cognitive rehabilitation	Prefrontal cortices	Seoul Computerised Neuropsychological Test

APPENDIX II

MRI Patient/Volunteer Checklist

It is ESSENTIAL for your SAFETY that you answer ALL questions

Surname:…… First Name:……

Date of Birth:…… Weight:…….. Height:……

Please ASK if you are unsure about ANY of the questions	Yes	No	Staff use
1) Have you *ever* had a **heart pacemaker**, **pacing wires** or internal **heart monitor**?	☐	☐	
2) Do you have **aneurism clips** in your head (from a surgical procedure)?	☐	☐	
3) Do you have a **deep brain stimulator** or **neurostimulator**?	☐	☐	
4) Do you have a **cochlear implant**?	☐	☐	
5) Do you have a **programmable shunt** (e.g. for hydrocephalus)?	☐	☐	
6) Do you have an **artificial heart valve** or **coronary stent**?	☐	☐	
7) Do you have any **metal implants** (e.g.joint replacements, pins, wires)?	☐	☐	
8) Do you wear **dentures**, **dental plate** or **brace**?	☐	☐	
9) Do you have a **drug delivery device** (e.g. insulin pump)	☐	☐	
10) Have you *ever* had any **shrapnel** or **metal fragments** in your body or eyes?	☐	☐	
11) Are you wearing a medicinal **patch**?	☐	☐	
12) Do you have any **artificial limb**, **calliper** or **surgical corset**?	☐	☐	
13) Are you wearing a **hearing aid**?	☐	☐	
Questions 14 & 15 for women only:			
14) Is it possible that you could be **pregnant**?	☐	☐	
15) Are you **breast-feeding**?	☐	☐	
16) DO YOU HAVE ANYTHING METAL WITHIN OR ABOUT YOUR BODY?	☐	☐	

Signature: …… **Date:** …… **Contact Number:** ……..

Name of person filling form (if not Patient/Volunteer):

……

Relationship to Patient ……..

DO NOT take metal objects into the examination room. This includes:

Keys, Coins, Watches, Jewellery, Mobile Phones, Hairgrips & Slides, Hearing Aids

Please store these and other valuables in the lockers provided

For your own safety please change into the clothing provided

For your information:

Loose metal objects	Can fly into the magnet like missiles
Pacemakers	May not work properly in the MRI Room
Travel/bank cards	May be erased if brought into the MRI Room
Watches	May be damaged by the magnet

Checked by:

Name: …… Signature: …… Date: ……

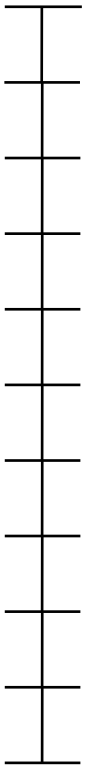
APPENDIX III

VAS

Can you please rate how much

you feel motivated to engage in this research, right now?

MAX



MIN

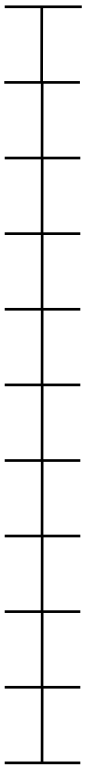
APPENDIX IV

VAS-2

Can you please rate how much

you feel motivated to engage in this research, right now?

MIN



MAX

APPENDIX V

tDCS Side Effects Questionnaire

(adapted from Brunoni et al., 2011)

Initials: Date:

Session n.

Do you think you received any stimulation? Yes/No

Did you experience any of the following symptoms/side effects?	**Enter a value (1-4)** **1. Absent** **2. Mild** **3. Moderate** **4. Severe**	**If present, is this related to tDCS?** **1. None** **2. Remote** **3. Possible** **4. Probable** **5. Definite**	**Notes**
Headache			
Neck pain			
Scalp pain			
Tingling			
Itching			
Skin redness			
Sleepiness			
Trouble concentrating			
Acute mood change			
Other (specify)			

APPENDIX VI

Normality tests for healthy controls

Output from the Kolmogorov-Smirnov test below.

Sleep & Motivation

Sleep REAL: D(39)=.252, p=.000; Sleep SHAM: D(39)=.199, p=.000

Motivation REAL: D(39)=.137, p=.062; Motivation SHAM: D(39)=.187, p=.001

Vigilance task

Younger adults

RT REAL: D(18)=.130, p=.200; RT SHAM: D(18)=.167, p=.199

CV REAL: D(18)=.100, p=.200; CV SHAM: D(18)=.173, p=.161

Accuracy REAL: D(18) = .144, p=.200; Accuracy SHAM: D(18)=.244, p=.006

Tot errors REAL: D(18)=.144, p=.200; Tot errors SHAM: D(18)=.244, p=.006

Omissions REAL: D(18)=.414, p=.000; Omissions SHAM: D(18)=.453, p=.000

FA REAL: D(18)=.135, p=.200; FA SHAM: D(18)=.247, p=.005

Older adults

RT REAL: D(21)=.114, p=.200; RT SHAM: D(19)=.146, p=.200

CV REAL: D(21)=.141, p=.200; CV SHAM: D(21)=.119, p=.200

Accuracy REAL: D(21) = .235, p=.004; Accuracy SHAM: D(21)=.204, p=.022

Tot errors REAL: D(21)=.236, p=.004; Tot errors SHAM: D(21)=.204, p=.022

Omissions REAL: D(21)=.496, p=.000; Omissions SHAM: D(21)=.321, p=.000

FA REAL: D(21)=.256, p=.001; FA SHAM: D(21)=.169, p=.119

Reverse score log-transformation was performed on accuracy.

For total errors, omission and commission errors, a constant was added before log-transforming, as some data tended to 0.

N-back task

Younger adults

RT REAL: D(36)=.104, p=.200; RT SHAM: D(36)=.101, p=.200

Accuracy REAL: D(36) =.179, p=.005; Accuracy SHAM: D(36)=.156, p=.028

Omissions REAL: D(36)=.179, p=.005; Omissions SHAM: D(36)=.156, p=.028

FA REAL: D(36)=.139, p=.075; FA SHAM: D(36)=.139, p=.075

Older adults

RT REAL: D(38)=.075, p=.200; RT SHAM: D(38)=.072, p=.200

Accuracy REAL: D(38)=.087, p=.200; Accuracy SHAM: D(38)=.107, p=.200

Omissions REAL: D(38)=.087, p=.200; Omissions SHAM: D(38)=.107, p=.200

FA REAL: D(38)=.136, p=.072; FA SHAM: D(38)=.801, p=.000

Reverse score log-transformation was performed on accuracy.

For omission and commission errors, a constant was added before log-transforming, as some data tended to 0.

APPENDIX VII

Pilot study

Introduction

Before commencing recruitment for the brain stimulation study, the computer task that was developed to track vigilant attention abilities and described in detail in Chapter IV, Section 3.3.1, was test piloted for feasibility and tolerability in patients. This small-scale tDCS-free pilot study was also carried out to ensure that the task would be able to elicit a vigilant decrement in the appropriate clinical population (i.e., a worsening in performance with increasing time-on-task), which could then be targeted by brain stimulation during the full-scale research project.

Methods

Three first-ever right-hemispheric stroke patients with neglect (22, 61 and 86 and years old, F=1, all right-handed) took part in the pilot test. They provided written informed consent before taking part in the study. They received practice runs of the vigilance task prior to data collection, until they were able to perform 10 consecutive trials at 100% accuracy. The vigilance task was then performed for 15 minutes, following the procedure delineated in Chapter IV, Section 3.3.1. Crucially, no breaks were allowed, and patients performed the 15 minutes task without interruptions.

Mean RT, target sensitivity (d') and total number of errors made by each patient were computed as described in Chapter IV, Section 3.4. No statistical analysis was performed at this stage, considering the probable variability in the patient group and the small numbers.

Results

All patients were able to complete the task and no issue was encountered during task administration.

Overall performance

Mean RT and target sensitivity (d') for each patient are displayed in Figure A.1. Patients performed the task at very different levels of target discriminability, with one patient (P1, shown in magenta colour) performing the task at a very low d'. No patient performance was at ceiling.

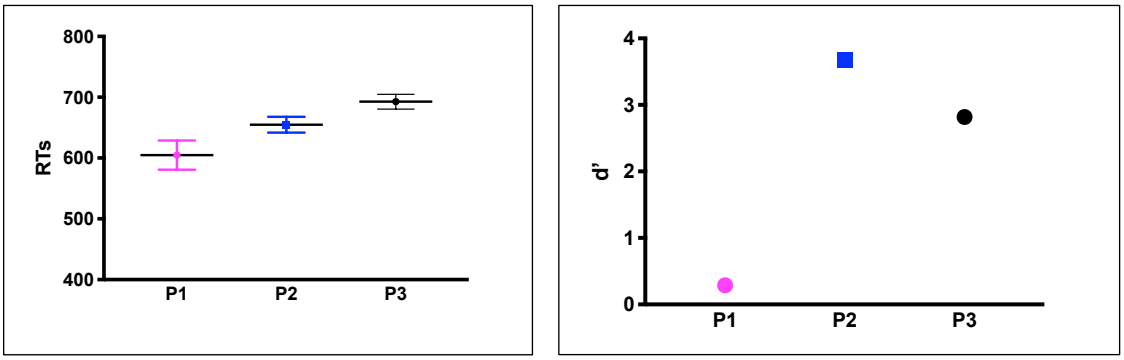


Figure A.1: Performance on the vigilance task for three stroke patients
Left panel: Mean RT (SEM) for each patient. Right panel: Average target sensitivity (d') for each patient.

The total number of errors made by each patient is shown in Figure A.2. P1 made far more errors on this task.

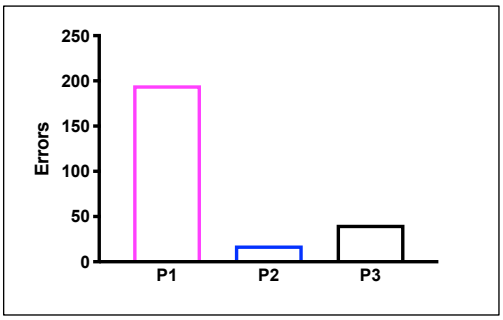


Figure A.2: Number of errors made on the vigilance task by each patient

Decremental performance

Changes in performance with increasing time-on-task were explored by dividing the task into 3 time-epochs, and calculating the outcome measures discussed above for each epoch.

Reaction times seemed to increase over time for one patient, whereas a speeding up of RT over time was observed for the other two (Figure A.3).

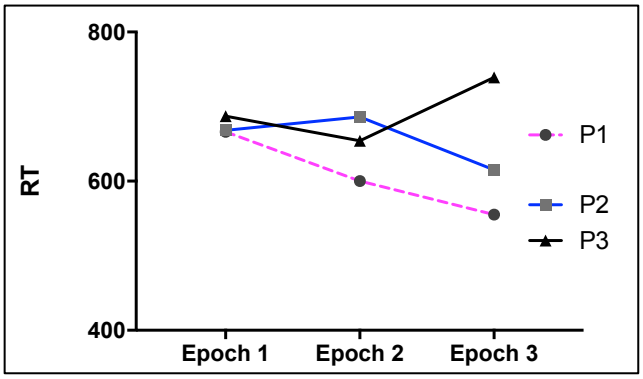


Figure A.3: RT across epochs on the vigilance task for each patient

The number of errors patients made on the task seemed to increase towards the end of the task (Figure A.4).

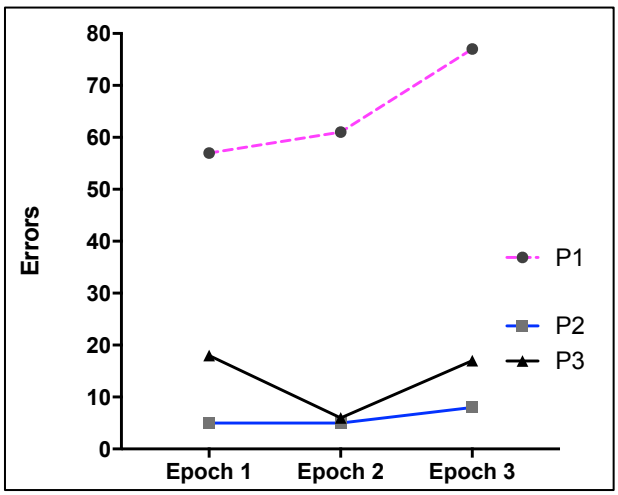


Figure A.4: Number of errors made by each patient with increasing time-on-task

A drop in target sensitivity seemed to take place towards the end of the task (Figure A.5).

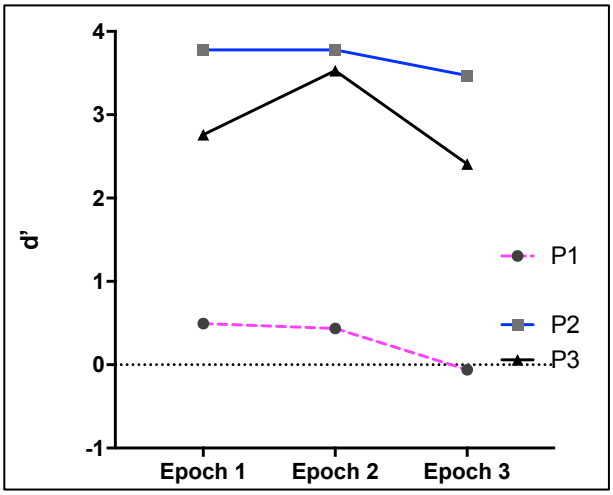


Figure A.5: Vigilance decrement for three stroke patients
Target sensitivity appears to decline with increasing time-on-task.

Discussion

Patients engaged well in the experiment. The paradigm used for the pilot study was considered able to elicit a vigilance decrement in the ability to discriminate targets from distractors in a sample of three chronic patients affected by right-hemispheric stroke.

APPENDIX VIII

Normality tests for patients

Output from the Kolmogorov-Smirnov test below.

Sleep (n=22) & Motivation (n=21)

Sleep REAL: D(22)=.165, p=.123; Sleep SHAM: D(22)=.151, p=.200

Motivation REAL: D(21)=.226, p=.007; Motivation SHAM: D(21)=.203, p=.024

VIGILANCE TASK (n=22)

RT REAL: D(22)=.134, p=.200; RT SHAM: D(22)=.110, p=.200

CV REAL: D(22)=.107, p=.200; CV SHAM: D(22)=.120, p=.200

Accuracy REAL: D(22)=.224, p=.005; Accuracy SHAM: D(22)=.243, p=.002

Tot errors REAL: D(22)=.224, p=.005; Tot errors SHAM: D(22)=.243, p=.002

Omissions REAL: D(22)=.235, p=.003; Omissions SHAM: D(22)=.239, p=.002

FA REAL: D(22)=.231, p=.003; FA SHAM: D(22)=.249, p=.001

Reverse score log-transformation was performed on accuracy. For errors, a constant was added before log-transforming, as some data tended to 0.

Levene's test for homogeneity of variance was also used to ensure variances were equal under real and sham tDCS for RT [F(1,42)=.000, p=.999], CV [F(1,42)=.001, p=.972], accuracy [F(1,42)=.467, p=.498], total errors [F(1,42)=.601, p=.442], omissions [F(1,42)=.271, p=.605] and false alarms [F(1,42)=.186, p=.669].

STAR CANCELLATION

Score REAL: D(44)=.284, p=.000; Score SHAM: D(44)=.319, p=.000

Asymmetry REAL: D(44)=.279, p=.000; Asymmetry SHAM: D(44)=.349, p=.000

Proc. speed REAL: D(44)=.286, p=.000; Proc. speed SHAM: D(44)=.215, p=.000

Cut-off score REAL: D(44)=.270, p=.000; Cut-off score SHAM: D(44)=.297, p=.000

Cut-off Asym. REAL: D(44)=.310, p=.000; Cut-off Asym. SHAM: D(44)=.336, p=.000

MESULAM CANCELLATION

Score REAL: D(44)=.245, p=.000; Score SHAM: D(44)=.247, p=.000

Asymmetry REAL: D(44)=.266, p=.000; Asymmetry SHAM: D(44)=.256, p=.000

Proc. speed REAL: D(44)=.184, p=.001; Proc. speed SHAM: D(44)=.217, p=.000

Cut-off score REAL: D(44)=.149, p=.015; Cut-off score SHAM: D(44)=.144, p=.023

Cut-off Asym. REAL: D(44)=.129, p=.065; Cut-off Asym. SHAM: D(44)=.159, p=.007

LINE BISECTION

Deviation REAL: D(44)=.237, p=.000; Deviation SHAM: D(44)=.227, p=.000

APPENDIX IX

t-DCS side effects questionnaire, scanner version

RUN 1

Do you think you received stimulation? YES NO

Can you please rate out of 5

(1= nothing, 2= mild, 3= moderate, 4= strong, 5= unbearable)

the following sensations:

Itching

Pain

Metallic Taste

Burning/ Tingling

Anxiety

Any other

RUN 2

Do you think you received stimulation? YES NO

Can you please rate out of 5

(1= nothing, 2= mild, 3= moderate, 4= strong, 5= unbearable)

the following sensations:

Itching

Pain

Metallic Taste

Burning/ Tingling

Anxiety

Any other