An exploration of the mechanisms of action of transcranial direct current stimulation (tDCS) in mood and anxiety disorder research

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Submitted for the degree of D.Phil.

University of Oxford, Trinity Term 2016

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But there's no sense crying over every mistake.
You just keep on trying 'til you run out of cake.
And the science gets done,
And you make a neat gun,
For the people who are still alive.

GLaDOS

Abstract

The difficulty in treating mood and anxiety disorders has brought about renewed clinical interest in alternative treatments, such as transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC). However, the optimal parameters for stimulation and underlying mechanisms of action are unclear. Psychiatric treatments have acute effects on emotional processing which predict later therapeutic action. Such effects have been proposed as cognitive biomarkers for screening novel treatments for depression and anxiety disorders. In this thesis the proposed cognitive, behavioural and neural underpinnings of mood and anxiety disorders are explored using tDCS of the DLPFC in humans.

This is investigated with a series of studies which measure the acute effects of tDCS on emotional processing relevant to depression and trait anxiety or anxiety disorders. An initial investigation in healthy volunteers revealed an anxiolytic like effect (reduced threat vigilance) of a single session of tDCS on a behavioural test of proven clinical relevance. In addition, stimulation parameters were refined for future studies. The subsequent two behavioural investigations combined tDCS with an attentional bias modification (ABM) training paradigm. However, no bias modification was achieved in these studies and predictably, no subsequent effects on attentional bias, mood/ trait anxiety or worry were observed. The first of the ABM investigations included a measure of cortisol awakening response (CAR) and it was found that a single session of tDCS (regardless of ABM condition) reduced subsequent CAR in healthy volunteers, also suggesting an anxiolytic effect. The final study, in a sample of trait anxious females, used functional imaging to reveal that tDCS of the DLPFC increased frontal activation and

reduced amygdala response to fearful face distractors during low cognitive effort. This provides the first causal evidence that modulating activity directly in the DLPFC inhibits amygdala response to threat in humans, and provides a potential neural mechanism for the previous reduction in vigilance to threat, and also for the efficacy of tDCS in the treatment of depression and anxiety disorders.

The evidence from this thesis puts forward an anxiolytic-like effect of frontal tDCS on cognitive, neural and adrenal biomarkers relevant to clinical anxiety disorders, and indicates a potential cognitive mechanism (reduced fear vigilance) and an underlying neural mechanism (increased top-down control of amygdala response) that may partially mediate the reported clinical efficacy of prefrontal tDCS in the literature.

Acknowledgements

There are an obscene number of people who have helped bring this thesis to fruition and I feel very fortunate to reflect on them. I'll start off with my supervisors, thanks to Cath for answering my cold email four years ago and allowing me the freedom to pursue my own program of research, whilst always leveraging her wisdom into my designs. To Jacinta, who taught me so much about critical thinking, writing and paying attention to detail and also for dragging me kicking and screaming through the dark days of early imaging analysis. It must have been a tough job and I'm very grateful for all the time she spent with me. I was also very lucky to work with some generous collaborators and students including Mike, Phil, Pat, Sonia, Laleh and Ntiriwa.

The lack of funds for my write up and an unrealistic notion of our ability to cope without a home led Lewis and I to 'couch surf' for the last eight months. The generosity of our friends has been overwhelming through this and I am compelled to list everyone who housed us during this time. A huge thanks to Chris and Rachel in Southfield Park, Rob, Alex and Lego in Bootle and Great Dunmow, John, Rah and Phoenix in Leytonstone and all at the Florence Villa, Stacey and Thom in Wanstead, Sarah in Catford, Beth and Tom in Croydon, Y-Ling and Hector on Bermondsey St, Tompkins at Queens, Martin and Michelle in Boston, Betty on Ardlea Rd, Alex and Laura on Iffley Rd, Chris, Sofie and Ned in Margate, Matt and Sana on Rectory Rd, Ruth, Woodie and Seren in Walthamstow, Kim, Paul and Louise in Canary Wharf, Shauna in Calais, Lisa on Alexandra Rd, Saul in Leyton, Charlotte in Oxford, Reuben in Bethnal Green and Alfie and Sam in London Fields. Although we've had no house of our own, being with family and friends has made us feel like we have a home and we can’t wait to host all these great characters in Boston.

To my family, who have given so much support to Lewis and I, even when we made choices that they really didn’t understand. In particular to my mam Betty and my in-laws Jen and Eddy, whose belief in us and financial support were crucial to us surviving the last few years. My brother Martin and his wife Michelle have showed us that an alternative lifestyle is possible and their support, both emotional and financial has surpassed any expectation. They are not only my family but two of my best friends and I'm so excited about our next adventure in Boston together. I'd also like to mention my dad. I wouldn't

be writing this thesis had he not continuously left piles of books in my bedroom when I was a child.

To my Oxford friends, thanks so much for making my DPhil such a fun experience. I'll never forget punting down the Cherwell in the Mayday morning sun. Especially to Y-Ling, whose friendship and constant encouragement kept me going. Chris has been such a supportive friend and helped me endlessly with housing, scanning, moving house, storage and moral support (and wine). Matthew and Charlotte also get a special mention for the mutual support and humour at the Warneford until the end.

To the artists, particularly the Sh*t-faced Shakespeare cast and crew. Thank you so much for giving me something to obsess about outside of my thesis (and to Louise and Beth for loaning their brains to my research). I am completely in love with making theatre happen with this magnificent bunch and I'm so grateful to have been along for the ride that has been the last four years. I'm indebted to artists of all kinds for helping me to understand the world a bit more, in particular to David Bowie, whose music accompanied much of this write up.

Finally… Lewis. Thanks for teaching me about lifelong learning, trying to leave something behind and for just being so unbelievably brilliant that I couldn't bear to be your wife and an accountant for the next 40 years. I feel like I can do anything when I'm with you and I hope that our lives will always be the adventure that you deserve.

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Long Abstract

The difficulty in treating mood and anxiety disorders has brought about clinical interest in alternative treatments, such as transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC). However, the optimal parameters for stimulation and underlying mechanisms of action are unclear. Psychiatric treatments have acute effects on emotional processing which predict later therapeutic action. Such effects have been proposed as cognitive biomarkers for screening novel treatments for depression and anxiety disorders.

Chapter One presents a review of the cognitive, behavioural and neural underpinnings of depression, trait anxiety and anxiety disorders, examined from the perspective of the cognitive neuropsychological model of antidepressant action. In particular, attentional biases and their modification as a mechanism of antidepressant/anxiolytic action are proposed as a key measure for this body of work. Non-invasive brain stimulation is proposed as a treatment for depression by reviewing clinical work to date and a parameter driven literature review follows, setting up initial parameters for the first experimental design.

Chapter Two presents an experiment which assessed the effect of tDCS on a battery of emotional processing measures sensitive to antidepressant action. To refine optimal stimulation parameters, DLPFC stimulation using two common electrode montages was compared to sham. 60 healthy volunteers received 20 minutes of active or sham DLPFC stimulation, before completing computerised emotional processing tasks, including a dot probe measure of vigilance to threat. Relative to sham stimulation,

participants receiving simultaneous anodal stimulation of left DLPFC and cathodal stimulation of right DLPFC (bipolar-balanced montage) showed reduced vigilance to threatening stimuli. There was no such significant effect when the cathode was placed on the supraorbital ridge (bipolar-unbalanced montage), thus refining parameters for future studies. There were no effects of tDCS on other measures of emotional processing. This significant reduction in fear vigilance is similar to that seen with anxiolytic treatments in the same cognitive paradigm. The finding that DLPFC tDCS acutely alters the processing of threatening information suggests a potential cognitive mechanism that could underwrite treatment effects in clinical populations.

Chapters Three and Five present experiments which assessed the effect of tDCS on attentional bias modification, first in a sample of 80 healthy volunteers and then in a trait anxious sample of 69 otherwise healthy volunteers. Participants received 20 minutes of bipolar balanced tDCS while they carried out a computerised attentional bias modification (ABM) training procedure. Measurements of attentional bias to threat and a lab based stressor were used to detect an effect of tDCS/ABM. These experiments did not provide evidence of attentional bias modification.

Chapter Four presents an additional assessment of the effects of tDCS/ABM on stress using measurements of cortisol awakening response (CAR), taken from the same healthy volunteer sample as Chapter Three. Measurements of CAR were taken the morning before the tDCS/ABM intervention, the morning after and also at a 28 day follow up. A single session of tDCS (regardless of ABM condition) was found to reduce subsequent cortisol awakening response (CAR) on the morning after stimulation. There were no interactions between tDCS and ABM on any of these follow up measures of CAR.

The direction of change is similar to acute reductions in cortisol seen after a single session of tDCS in healthy volunteers. This reduction in CAR is also similar to effects seen with antidepressant drug treatments. It is possible that this decreased CAR could be a biological correlate of the reduction in threat vigilance seen in Chapter Two and/or antidepressant effects of tDCS in clinical trials. Taken together, these results suggest that CAR may be a potentially useful biomarker to consider for the prediction and measurement of tDCS efficacy in clinical groups.

Chapter Six utilises data from the previous experimental studies to conduct a pooled data analysis of tDCS and ABM with greater statistical power. This analysis revealed no further effects of tDCS or ABM.

The study in Chapter Seven used tDCS of the DLPFC to explore the effects on amygdala response during presentation of threat using fMRI. 16 anxious females received both active and sham tDCS of the DLPFC and carried out an attentional control task with emotional distractors. Stimulation of the DLPFC with tDCS increased frontal activation and reduced amygdala response to fearful face distractors during low cognitive effort compared to the sham stimulation. This provides the first causal evidence that modulating activity directly in the DLPFC inhibits amygdala response to threat in humans. In so doing, this provides a potential mechanism for the effects of tDCS in reducing vigilance to threat and also for the efficacy of tDCS in the treatment of depression and anxiety disorders.

Finally, Chapter Eight summarises the evidence and puts forward an anxiolytic-like profile of frontal tDCS, supported by behavioural and neuroimaging data, and measures of

adrenal function. This is based on the current phase I results, which reveal an anxiolytic-like effect of frontal tDCS on cognitive and neural biomarkers relevant to clinical anxiety disorders, and indicate a potential neurocognitive mechanism (reduced fear vigilance) and an underlying neural mechanism (increased top-down control of amygdala response) that may partially mediate the reported findings of clinical efficacy of prefrontal tDCS in the literature.

List of key abbreviations

ABM	attentional bias modification
ANOVA	analysis of variance
ASL	arterial spin labelling
CFS	continuous flash suppression
CNS	central nervous system
cAMP	cyclic adenosine monophosphate
DLPFC	dorsolateral prefrontal cortex
ECT	electroconvulsive therapy
ETB	emotional test battery
FERT	facial expression recognition task (behavioural version)
fMRI	functional magnetic resonance Imaging
GABA	gamma-amino butyric acid
HPA	hypothalamic-pituitary-adrenal
IAPS	international affective pictures series
mA	milliamp
MADRS	Montgomery–Åsberg depression rating scale
ms	millisecond
MDD	major depressive disorder
PANAS	positive and negative affective schedules
SEM	standard error of the mean
STAI	state-trait anxiety inventory
SSRI	selective serotonin re-uptake inhibitor
NRI	norepinephrine re-uptake inhibitor
tDCS	transcranial direct current stimulation

Chapter 1

General Introduction

1.1. MDD and anxiety disorders

1.1.1. Depression

An episode of depression serious enough to require treatment occurs in about one in four women and one in ten men at some point during their lives (1). Major Depressive Disorder (MDD) is the leading cause of disability worldwide in terms of total productive years lost (2). Even so, this pervasive condition still attracts much stigma and identifying effective treatment can be difficult - from initial diagnosis and access to services through to determining the most appropriate intervention for the individual.

Risk factors for MDD include female gender, employment in the home, unemployment, disability, absence of a life partner, low educational level and low income. Comorbidity with anxiety disorders and substance abuse disorders is also common (3, 4). There is evidence for genetic risk factors for MDD (5), though the power to detect specific genes is limited (6), perhaps because of genetic loadings on different symptoms of MDD rather than a single dimension of genetic risk (7). MDD is diagnosed using a standardised clinical interview (the most recent being the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, DSM-5 (8)), which takes common features of MDD into account (e.g. low mood, loss of pleasure, loss of appetite, sleep disturbance, psychomotor symptoms, fatigue and thoughts of death) along with the level of distress or loss of functioning associated with these symptoms. In research, levels of depression are

measured using rating scales such as the Hamilton Depression Rating Scale (HDRS) (9), the Beck Depression Inventory (BDI) (10) and the Montgomery-Asberg Depression Rating Scale (MADRS) (11). All of these measures rely heavily on self-report and there is a complete lack of objective biological tests to guide treatment choices, reflecting our inadequate understanding of the biological basis of MDD.

The first available antidepressant drugs block the re-uptake of norepinephrine (NE), serotonin (5-HT) and dopamine (DA) by the presynaptic neuron, which increases the availability of these neurotransmitters in the synapse and increases stimulation of the postsynaptic neuron (12). This led to the theory of depression known as the monoamine-deficiency hypothesis, which was further investigated with the inhibition of monoamine synthesis with tryptophan depletion (13, 14). This enabled the effect of decreased serotonin to be investigated as a cause for depression by depleting the amino acid precursor to serotonin in trials. However tryptophan depletion induced depression only in previously depressed patients, or those with a family history of depression and not in healthy controls (15). This suggests that the monoamine-deficiency hypothesis does not fully explain the symptoms of MDD.

Clinical interventions such as antidepressant drugs and cognitive behavioural therapy (CBT) can be effective in treating the symptoms of MDD. However, these treatments do not work for everyone, and even when they do work, relapse is common. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) programme of research incorporated nearly 4000 depressed patients and four sequential treatment routes involving; single medication, medication augmentation, medication switching and psychotherapy augmentation. Remission in each of the four treatment strategy steps was

31%, 37%, 14% and 13% progressively (16). It was found the more treatment steps that were pursued, the lower the remission rate (17), highlighting the need for alternative or additional treatments.

1.1.2. Anxiety disorders

Anxiety disorders are common worldwide, with a recent review estimating 1 out of 14 people meet diagnostic criteria at any point in time (18). The group of disorders include generalised anxiety disorder (GAD) (prolonged excessive worry, usually accompanied with other nonspecific physical/psychological symptoms), phobias (disproportionate fear of an object or situation, e.g. spiders, closed spaces), panic disorder (unexpected/ overwhelming feelings of anxiety, accompanied with physical symptoms) and separation anxiety disorder (fear and nervousness of being away from home and loved ones). The DSM-5 lists common symptoms including anxiety/worry which is difficult to control, restlessness, fatigue, difficulty concentrating, muscle tension and sleep disturbance. Thus, anxiety disorders cause a significant amount of distress, with 70% of reported suicide attempts in the United States occurring in patients who had a previous anxiety disorder diagnosis (19). In addition there is a high incidence of chronic unemployment, drug and alcohol abuse within this patient group (20). Anxiety disorders are highly comorbid with MDD (21) the incidence of anxiety disorders in adolescents has been found to substantially increase the risk of depression later in life (22). As with MDD, gender effects are seen in anxiety disorder prevalence, for example women are twice as likely to have GAD than men (4). Other risk factors for anxiety disorders include low socioeconomic status and childhood trauma (23). Anxiety disorders are also diagnosed using a standard clinical interview and measured using rating scales such as the State-Trait Anxiety Inventory (STAI) (24) and the Hospital Anxiety and Depression Scale (HADS) (25) and thus present

with similar problems to depression in terms of their reliance on subjective self-report and lack of a direct biological measure.

Anxiety disorders are highly comorbid with depression, with many overlapping symptoms. However, there are some distinguishing features between the two types of disorder. Anhedonia, the inability to experience pleasure, is symptomatic of depression and not trait anxiety or anxiety disorders. Additionally, trait anxiety or anxiety disorders are characterised by feelings of helplessness, while depression is associated with feelings of hopelessness (26). Anxiety disorders are commonly treated with antidepressant/ anxiolytic drugs and/ or several forms of psychotherapy. However, response rates in clinical trials range around 30-50% (27, 28), reflecting the need for alternative treatments.

1.1.3. Dimensional framework

The overlapping symptoms of MDD, anxiety disorders and other mental conditions have brought about the need for research to explore the underlying features and systems which characterise these disorders. The Research Domain Criteria (RDoc) (29) initiative is an example of progress in this direction. The RDoc consists of a matrix with functional constructs of behaviour (e.g. fear, working memory, sleep), which can be characterised at the level at which they are measured (e.g. genes, circuits, behaviours). These constructs are then summarised into five domains (i) negative valence systems, (ii) positive valence systems, (iii) cognitive systems, (iv) social processes, and (v) arousal and regulatory systems. This provides researchers with an evolving dimensional framework which will help identify new targets for treatment development, detect subgroups for treatment selection, and provide a better match between research findings and clinical decision making (30).

1.1.4. Treatments

1.1.4.1. Pharmacological treatments

Antidepressant drugs are the first line of treatment for MDD recommended by the National Institute for Clinical Excellence (NICE), with administration of selective serotonin re-uptake inhibitors (SSRIs) such as fluoxetine, citalopram and sertraline, or norepinephrine re-uptake inhibitors (NRIs) such as reboxetine, or serotonin–norepinephrine re-uptake inhibitors (SNRIs) such as venlafaxine and duloxetine. These rationally designed drugs act by selectively increasing the level of extracellular serotonin and/or norepinephrine available to bind to the postsynaptic receptor by inhibiting its re-uptake into the presynaptic cell. These selective drugs were designed to have similar efficacy to the non-selective drugs previously used but with fewer side effects, resulting in a more favourable risk-benefit ratio. Most clinical guidelines suggest that it is only when a selective drug fails to produce a response that older, non-selective drug treatments such as tricyclics (which affect multiple neurotransmitters) should be administered (31). In addition bupropion, a drug which inhibits the re-uptake of dopamine and norepinephrine has shown a similar level of efficacy as SSRIs (32).

Antidepressants have been shown to improve response (defined as a 50% reduction in HDRS/MADRS scores) and remission (defined as HDRS < 8 or MADRS < 11-13) compared to placebo (31). Placebo response in depression is a problem which affects research; a meta-analysis of 75 published short-term randomised controlled trials (RCTs) showed a response rate of 50% for antidepressants compared to 30% for placebo (33). In addition, there is evidence of a publication bias problem, with an analysis of 74 placebo controlled RCTs registered with the US Food and Drugs Administration (FDA) showing

that 31% (primarily negative) of these were not published (34). These research problems raise questions about the efficacy of drug treatment for the treatment of depression. However, the lack of alternatives means that it remains the most common treatment strategy.

Anxiety disorders are commonly treated with antidepressant medications such as SSRIs, administered in a similar way as when treating depression, and with similar problems to those outlined above. In addition, anxiolytic medications such as benzodiazepines are used to treat acute symptoms in anxiety disorders. There is evidence to support longer term treatment of GAD with benzodiazepines in some cases (27), although there is some reluctance from clinicians because of the risk of dependence. Benzodiazepines such as diazepam enhance the effect of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor, resulting in anxiolytic effects (along with sedative properties). Treatment resistant patients may be offered treatment (either as an alternative to antidepressants or as an adjunct) with second generation antipsychotic medications such as quetiapine, the use of which is supported by clinical trial data (35). These drugs have effects on a number of neurotransmitters, including norepinephrine (NE), serotonin (5-HT) and dopamine (DA) and these come with risks of metabolic abnormalities. Because of these risks and the non-specificity of the pharmacological approaches to anxiety disorders, psychological interventions are a more popular treatment route.

1.1.4.2. Psychological treatments

Depression and anxiety disorders are treated with a number of psychological interventions such as cognitive behavioural therapy (CBT), non-directive supportive treatment (SUP),

behavioural activation treatment (BA), psychodynamic treatment (DYN), problem-solving therapy (PST), interpersonal psychotherapy (IPT) and social skills training (SST), as summarised in a review of 53 clinical studies (36).

CBT aims to modify a patient's dysfunctional beliefs through cognitive restructuring (37) and, in some cases, encompasses additional components such as behavioural activation and coping skills. SUP is a catch all term for any unstructured therapy or counselling without specific psychological techniques, this may include reflection (38) as a key component and is based on the belief that relief from personal problems may be achieved through discussion with others. BA is based on the core principles of registration of pleasant activities and increase of positive interactions with the patient's environment (39), and could include some social skills training. Short term DYN has the central objective of enhancing the patient's understanding, awareness and insight about repetitive conflicts in their emotional and life experiences. PST is a step-by step approach involving the definition of personal problems, the generation multiple solutions and selection of the best solution (40). IPT is a brief, manual based therapy that focuses solely on interpersonal issues in depression (41). Finally, SST teaches skills that help patients build and retain social and interpersonal relationships.

The meta-analysis, mentioned above, of 53 studies found that there is no superior treatment between these aforementioned seven psychological interventions, for mild to moderate MDD. However, in general, IPT was found to be somewhat more efficacious and BA was found to be slightly less efficacious, although long term outcomes are not known. Drop-out rates were higher for CBT and lower for PST in this analysis (36).

Clinical trials support the treatment of anxiety disorders with a number of psychological interventions such as CBT, DYN, mindfulness based therapy and applied relaxation therapy (26). CBT has the strongest evidence (42) for the treatment of anxiety disorders and because of this is usually the first line intervention. CBT targets the maladaptive level of perceived danger that patients experience in their environment by using cognitive restructuring to help them understand that worry is not beneficial, exposure therapy to demonstrate the ability to change behaviours and relaxation training to provide a tool for coping with stressful situations. After a course of CBT for an anxiety disorder a reduction in intolerance of uncertainty is an important mediator of treatment outcome (43). Mindfulness based therapy is derived from ancient Buddhist and yogic practices, with the core concepts of self-regulation of attention and experiencing the present moment non-judgementally, by means of slow and deep breathing. A meta-analysis of 39 studies found strong positive effects of mindfulness based therapies on both mood and anxiety disorder symptoms (44). Finally, applied relaxation therapy is a taught coping skill which enables the patient to relax rapidly and is useful in panic disorders and phobias, with long-lasting effects reported up to 19 months after treatment (45).

Advances in technology resulting in the increased availability of personal computers and smartphones have allowed therapies to be computerised and delivered online. Computerised CBT has shown efficacy in the treatment of depression and anxiety disorders (46) and provides obvious cost savings in terms of therapist time. Technology has also allowed the development of novel experimental treatments such as attentional bias modification training (47, 48). The ability to deliver these computerised interventions to large samples opens up new paths for clinical research, although with this come new problems of monitoring and adherence.

1.1.5. Treatment resistance and relapse

MDD is a recurrent condition, with 80% of people who have received treatment for depression having at least one further episode and a median of four episodes in their lifetime (31). In a 12 year follow up study of 431 MDD patients, depressive symptoms were present for 59% of the time with 15% of this classified as major depression and 22% of patients never being free of depressive symptoms during the entire follow up period (49). Anxiety disorders are also characterised by a chronic course, with CBT and drug interventions only reducing symptoms in 50% of patients (50). The definition of remission in an anxiety disorder is complicated due to the avoidance techniques used in phobias and the presence of normal life stress; this waxing and waning course indicates an actual recovery rate from standard treatments of around 30% (51). The distress caused to the individual and the socioeconomic cost of this unfilled treatment gap means there is potentially much to be gained in exploring novel methods of treating depression and anxiety disorders, which can be informed by greater knowledge of the cognitive neuroscience of these disorders.

1.2. The cognitive neuroscience of mood and anxiety disorders

1.2.1. Neural circuits

The brain areas most reproducibly found to be dysregulated in emotional disorders are the prefrontal cortex (PFC), subgenual cingulate cortex (SGC), the hippocampus and the amygdala (for a review, see (52) and (53)). The neural circuit approach to characterising depression and anxiety disorders often focuses on the interaction of frontal regions such as the dorsolateral prefrontal cortex (DLPFC) with limbic regions such as the amygdala.

1.2.1.1. Amygdala

The amygdala is one of the key brain regions implicated in the pathophysiology of depression and anxiety disorders. Mainly pre-clinical research in the 1980s established the amygdala's role in fear conditioning, with conditioned fear being mediated by the transmission of information to the amygdala and the control of fear reactions by way of output projections from the amygdala to the behavioural, autonomic, and endocrine response control systems located in the brainstem (54). It is thought that fear learning might be expressed through plasticity in the amygdala. Long term potentiation (LTP) and long term depression (LTD) are the cellular processes believed to underlie learning. In LTP, glutamate (the primary excitatory neurotransmitter in the brain) binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and depolarises the postsynaptic cell. This depolarisation allows glutamate to bind to the N-methyl-D-aspartate (NMDA) class of receptors. Calcium then flows into the cell through the NMDA channel and triggers a host of intracellular events that ultimately result in gene induction and synthesis of new proteins which help to stabilise changes over long periods of time (54, 55). LTD refers to any long term depression in synaptic transmission (56), which enables the relative strength of synaptic strengthening from LTP to be effective. Inactivation of the amygdala during fear conditioning, through focal amygdala infusions, has been shown to prevent the acquisition and expression of fear conditioning separately (57). An interesting difference between conditioned stimulus fear (e.g. a shock paired with an auditory tone) and contextual fear (e.g. when the rodent is put in the cage where the shocks were initially administered) is that the latter requires additional input from the hippocampus, reflecting sensory relay (58).

The turn of the 21st century brought about a wealth of human research on the amygdala and fear processing. The significance of the amygdala in linking emotion and attention was first highlighted over forty years ago (59) and subsequently fear perception was found to be impaired in patients with amygdala damage (60). However, research lagged until advances in the quality and availability of functional neuroimaging techniques during the last 20 years enabled cognition and emotion to be studied together. Functional imaging has shown amygdala activation in response to fearful faces (61), even when these are perceived without explicit knowledge (62), implicating a role for fast (subconscious) processing. There is a hypothesis that the amygdala receives processed output from thalamo-cortical sensory regions and also projects back to these regions to guide early processing stages, particularly in terms of detecting danger (54).

Depressed patients exhibit hyperactive amygdala responses to emotional information (63-65), as do patients with anxiety disorders (66, 67). Treatment with SSRIs has been shown to attenuate amygdala response to fearful faces in healthy controls (68, 69) and in depressed patients, both after treatment response (64) and before reported treatment response (70, 71). The latter studies indicate that the reduction in amygdala hyperactivity may be an important part of the mechanism of action of SSRIs in improving mood. However, this bottom-up fear response should be examined in the context of its interactions with other brain regions, such as the pre-frontal cortex.

1.2.1.2. Pre-frontal cortex and top-down control

Animal studies provide compelling evidence for the importance of the pre-frontal cortex (PFC) in regulating responses to threat via direct inhibition of the amygdala complex. The PFC acts to inhibit aversive associations established in fear conditioning, with PFC lesions

impeding (72), and conversely electrical stimulation of the PFC enhancing (73), the extinction of a conditioned response in mice and rats. Extinction is associated with plasticity changes in the cortico-amygdala pathway (74) as the PFC enables the learning of non-aversive associations to replace the previously conditioned aversive associations. In fact, persistence of electrical activity associated with learning or LTP in the PFC during extinction is associated with the maintenance of extinction learning in mice (75). Furthermore, electrical pre-stimulation of the PFC in rats and cats (76) specifically blocks or reduces subsequent response from amygdala output neurons. The results from these pre-clinical investigations provide the foundation for theoretical models of emotional dysfunction in disorders of depression and anxiety. As such, these disorders have been hypothesised to occur when there is a failure of cortical modulation of the lower-level limbic response to threat (77, 78). Consistent with this, these disorders are associated with an imbalance in activity levels of this cortico-limbic circuit in neuroimaging studies (79-82). However, there is currently no direct evidence that this circuit functions in the same way in humans as is reported in pre-clinical animal models, i.e. that frontal control regions, including the DLPFC, inhibit amygdala responses to threat.

The DLPFC is implicated in 'higher' cognitive functions, summarised by Smith and Jonides (83):

(i) Focusing attention on relevant information and processes and inhibiting irrelevant ones.

(ii) Scheduling processes in complex tasks, which requires the switching of focused attention between tasks.

(iii) Planning a sequence of subtasks to accomplish some goal.

(iv) Updating and checking the contents of working memory to determine the next step in a sequential task.

(v) Coding representations in working memory for time and place of appearance.

Central to its role in psychiatry is (i) inhibiting irrelevant information, with the DLPFC as a proposed top-down regulator of limbic responses to salient stimuli such as fearful faces. Along with a hyperactive amygdala, there is evidence that depressed patients have a hypoactive DLPFC (77). The PFC is activated during the stress response in healthy people, with maladaptive responses leading to an over activation of the hypothalamic-pituitary-adrenal (HPA) axis, a feature which may constitute an endophenotype for stress-related disorders (84). In the previously discussed study of attentional control in trait anxiety, Bishop et al. (85) showed the reverse pattern for the DLPFC and amygdala with high trait anxious subjects showing hyperactive amygdala and hypoactive DLPFC, further implicating the role of frontal cortex top-down control in the regulation of maladaptive limbic pathology.

1.2.1.3. Other components of the neural circuit

Transient sadness is associated with decreases in activity in prefrontal cortical regions and increases in limbic/ paralimbic regions including the SGC and the anterior insula in healthy volunteers. In addition, resolution of dysphoric symptoms is associated with the opposite pattern of activity (86). This suggests two further key regions which may advance our understanding of the cortico-limbic circuit.

The SGC has extensive projections to and from the orbitofrontal cortex, medial pre-frontal cortex, the insula and the amygdala (87). The SGC is overactive in MDD and

this activity is reduced with antidepressant treatment (86). The SGC is thought to mediate aspects of circadian regulation and cognitive processes such as learning, memory, motivation and reward (88). The SGC is the main target for deep brain stimulation (DBS) in treatment resistant depression (89), with a reduction in SGC activity associated with an improvement in symptoms (88).

The insula is unique in that it is situated at the interface of the cognitive, homeostatic, and affective systems, providing a link between stimulus-driven processing and brain regions involved in internal monitoring (90, 91). Reduced grey matter volume in the insula and the anterior cingulate cortex (ACC) is shown across a broad range of psychiatric diagnoses (92). Depressed patients have greater resting-state functional connectivity between the insula and the amygdala, SGC, and orbitofrontal cortex compared to healthy controls (93).

The cortico-limbic circuit is complex and its definition is still in its infancy. The individual components and their connections must be further elucidated in order to understand markers of treatment response, an important factor in increasing treatment success.

1.2.1.4. Neural biomarkers of treatment response

Key to the translation of a neural circuit of depression and anxiety disorders is identifying those activity patterns which can predict treatment response. Neuroimaging research has established some potential neural biomarkers which may be usefully employed to characterise responders and non-responders to different types of treatments. The ACC is implicated in studies using positron emission tomography (PET) and

electroencephalogram (for a review see (94)), with pre-treatment hyperactivity in this region predicting response across a range of treatments. Taking this a step further a recent study showed a treatment specific biomarker in right insula brain glucose metabolism, which, prior to treatment was hypoactive for subsequent responders to CBT and hyperactive for subsequent responders to the SSRI escitalopram (95). Neural biomarkers such at this will be critical to developing better targeted treatments for depression and anxiety disorders, which currently rely heavily on subjective measures and blunt stepwise administration of treatment tools according to broad diagnoses. Given their central role in emotional response and regulation, the prefrontal cortex and amygdala are promising regions to additionally test, in order to potentially establish new neural biomarkers.

1.2.2. Behavioural changes

1.2.2.1. Cognitive schema and biases

Beck (96) expanded upon his original description of the cognitive processes in depression, presenting six separable but overlapping models consisting:

(i) The *cross-sectional* model which states that the systematic negativity that pervades the cognitive processes is a necessary but not sufficient component of depression.

(ii) The *structural* model which stipulates that certain negatively based schemas are hyper valent in depression and shift the cognitive processes sufficiently to produce a systematic bias in the abstraction of data, interpretation, short-term memory, and long term memory.

(iii) The *stressor-vulnerability* model, which stipulates that specific patterns of schemas made a person sensitive to particular stressors.

(iv) The *reciprocal-interaction* model, which focuses on the ways that interaction with key figures is relevant to the predisposition, precipitation, aggravation, prolongation, and recurrence of depression.

(v) The *psychobiological* model, which views cognitive processes and biological processes as different sides of the same coin and integrates genetic, neurochemical, physiological, psychological, affective, and behavioural aspects.

(vi) The *evolutionary* model, which views depression as an atavistic mechanism or program that may have been adaptational in a prehistoric environment but is not adaptive in our current milieu.

Depressed patients typically show negative biases in perception and memory, for example greater recognition of sad faces and diminished recognition of happy faces, or increased memory for negative personal remarks compared to positive personal remarks. Such biases in emotional processing are believed to play a fundamental role in the maintenance of mood disorders (37). Anxiety disorders reliably present with attentional bias or vigilance towards threat (97), referring to differential attentional allocation towards threatening information compared to neutral information (98).

1.2.2.2. Vigilance to threat and attentional control in trait anxiety and anxiety disorders

Anxiety, fear and vigilance to threatening information are thought to serve an adaptive function. However, maladaptive anxiety forms the basis of anxiety disorders which cause distress and impair function (99). Trait anxiety and anxiety disorders are related to attentional control, with strong correlations between questionnaire measures of attentional control (measured using the Attentional Control Scale, ACS) and trait anxiety (measured

using the STAI-T) (100). Attentional control theory (101) states that state anxiety disrupts the balance between the two attentional systems; the goal directed (top-down) system influenced by expectation, knowledge and current goals, and the stimulus-driven (bottom-up) attentional system responding maximally to salient or conspicuous stimuli, with the disruption favouring the influence of the latter. The components of this disruption include facilitated attention to threat (102, 103), disability to disengage from threat (104) and attentional avoidance (105). This is shown behaviourally, associating trait/state anxiety or an anxiety disorder diagnosis with improved performance in dual task paradigm tasks with salient stimuli (106, 107), impeded pre-potent response inhibition in the Stroop task (108) and increased vigilance to threat in dot probe paradigms (109). This attentional control deficiency means that anxiety disorder patients are more vigilant towards, and thus distracted by, salient (fearful) stimuli. However, they lack the level of attentional control required to stay focused on the current task.

1.2.2.3. Mechanisms of action of antidepressant and anxiolytic drug action

Very little is understood about how the neurochemical effects of antidepressant drugs translate into a reduction in the clinical symptoms of mood disorders. Traditionally, it is assumed that following administration of drugs such as SSRIs or NRIs, the increased extracellular level of monoamines such as serotonin or norepinephrine resulting from the action of these drugs directly improves mood. However, mood is generally measured with subjective self-report questionnaires, which may fail to capture subtle changes. An unexplained feature of antidepressant drug action is that clinical improvement is not seen until treatment has continued for several weeks (110). Combining this with the phenomenon of negative biases, Harmer et al. (111) propose that the time-lag in mood improvement experienced when patients undergo treatment with an antidepressant is

explained by the (more swift) change in emotional processing with a reduction in negative bias. This shift of bias in a more positive direction is a proposed mechanism of action for mood improvement from antidepressant treatment. These changes in emotional processing are observed in healthy people and clinical groups. Trials with only a single dose of reboxetine (NRI) on healthy participants show an acute marked positive bias in facial expression recognition, emotional categorisation, and emotional memory, only two hours after taking the drug (112). Further evidence for this positive bias effect emerged from a clinical study by Tranter et al. (113) using randomised treatment with citalopram (SSRI) or reboxetine. Changes in emotional processing are compared to clinical outcome following six weeks of treatment. There is a significant increase in recognition accuracy of emotion following two weeks of treatment with either antidepressant. In addition, there is a significant correlation between the increased accuracy in recognition of happy faces over the first two weeks of treatment and clinical improvement after six weeks of treatment.

These results suggest that the psychoactive effects of antidepressant drugs do not directly improve mood. Instead, they provide a basis for improved mood through more positive processing of emotional and social stimuli. These early effects also show the potential to predict treatment response and may indicate the mechanism of action of treatments, thus increasing our understanding of how treatments work.

Acute effects on emotional processing relevant to trait/state anxiety and anxiety disorders are also seen with anxiolytic treatment. Vigilance to fearful-faces in a dot probe paradigm is attenuated acutely with a single dose of diazepam (114) and an initial session of a CBT intervention for panic disorder (115), which is also predictive of later treatment

response. This suggests a similar emotional bias mechanism of action for anxiety disorder treatment, driven by a more acute reduction of threat vigilance.

This growing body of evidence indicates that emotional processing is altered by antidepressant and anxiolytic treatment, in healthy volunteers and patients, and in the latter group can predict treatment response ahead of time (116). There is therefore potential to better understand mood and anxiety disorders through examining the cognitive features of treatment and the emerging neuroscientific evidence, thus developing a cognitive neuropsychological model of mood and anxiety disorder treatment, based on emotional biases.

1.2.2.4. Neural mechanisms of affective biases

Antidepressant treatment effects are associated with neural modulation in limbic and pre-frontal circuitry. Neuroimaging studies show that in depressed patients, functional brain activity in subcortical limbic areas (including the amygdala) are exaggerated in response to negative information and reduced in response to positive information (117). These areas are recruited during initial, evolutionarily primitive and automatic responses to emotion, in particular fear processing (54). In addition, depressed and anxious patients have increased amygdala activation to emotional stimuli (66, 118) and decreased prefrontal activation in response to cognitive tasks (119).

In terms of an individual's subjective emotional experience in a given environment, an important feature of emotional processing is attentional control and information filtering, which is thought to be a function of the PFC and ACC (120). This top-down control from frontal regions involves the conscious evaluation of emotional experience

which then influences automatic bottom-up responses from sub-cortical areas. Therefore an individual's subjective emotional response to environmental factors could depend on the relative strength and interaction between the top-down frontal cortical activity and the more automatic, sub-cortical activity. Harmer et al. (121) suggest that antidepressant drugs target the initial, subcortical processes whereas cognitive therapy may modulate the cortical processes of top-down control. This is supported with neuroimaging evidence comparing the two types of intervention (122), but needs further investigation.

Brain imaging studies reveal hypo-activation of the PFC in trait anxiety (85, 123, 124), which is thought to reflect deficient attentional control (125). This emotional neural circuit is associated with the cognitive features of anxiety disorders, with the amygdala facilitating attention and conversely the frontal cortex affecting both disability to disengage and attentional avoidance (126).

Amygdala responses to fearful faces are correlated with trait anxiety (85) in an attentional control paradigm. This finding adds to the cognitive load theory of processing (127) which states that there must be capacity in processing resources to enable salient stimuli (e.g. fearful faces) to capture attention. In this study the amygdala shows a selective response to fearful distractor stimuli in a letter search task when the task itself is easy (low perceptual load condition). When the letter search task is more difficult (high perceptual load condition), attentional resources are fully occupied and therefore salient distractor stimuli don't capture attention in the same way. Low anxious subjects have lower amygdala activation to fearful faces under conditions of low perceptual load than high anxious subjects. In addition, low anxious subjects have higher accuracy than high anxious subjects in this study, emphasising an attentional control deficit in trait anxiety.

The understanding of these cognitive features, alongside the neural circuits of depression and anxiety disorders, will be crucial for designing experiments which test and refine novel treatments for these conditions, such as non-invasive brain stimulation.

1.3. Non-invasive brain stimulation

1.3.1. History

The idea of stimulating the brain with electricity has been around for thousands of years and its history is summarised in (128). Earliest records describe Roman court physician Scribonious Largus using torpedo fish to cure headaches and gout in 43 AD, an early means of transcutaneous electrical nerve stimulation (TENS). In the 18th century the famous polymath Luigi Galvani invented the voltaic cell and carried out initial 'animal electricity' experiments. His nephew Giovanni Aldini took this further and published his *Essai theorique et experimental sur le galvanismse* (129), which reported experiments using electric 'therapy' to treat psychoses and melancholia, although his theatrical approach (see Fig. 1.3.1.1.) to the experiments may have contributed to the decline of clinical interest in non-invasive brain stimulation for over 100 years.

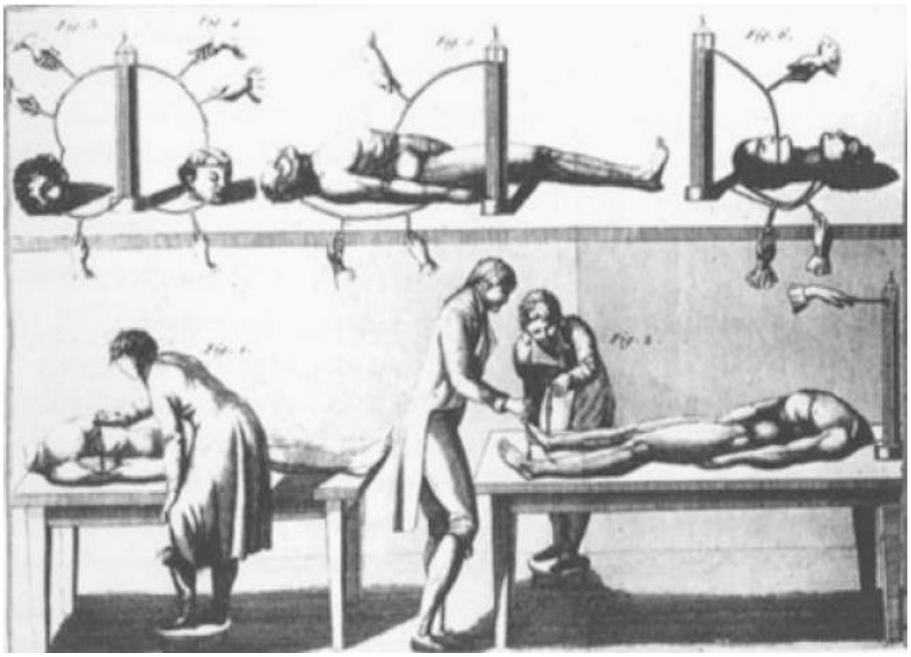

Figure 1.3.1.1. An illustration of Aldini's experiments in the early 19th century show him attempting to reanimate corpses (128).

Interest re-emerged in the 1960's with animal studies using direct current to show that weak electrical currents in magnitudes much lower than necessary for action potential could produce lasting effects on neuronal excitability in rats (130) and cats (131), laying the foundations for later non-invasive electrical stimulation protocols in humans, using battery powered devices.

1.3.2. Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is an established investigative tool in cognitive neuroscience. TMS utilises rapidly changing magnetic fields to induce an electrical current in the cortex and can be used to induce a transient interruption of normal brain activity in a relatively focal area (132), thereby creating a 'virtual lesion' (133). Repetitive transcranial magnetic stimulation (rTMS) utilises different frequencies of stimulation pulses, which, depending on the frequency, can be excitatory or inhibitory. RTMS applied to the left DLPFC has been approved by the US Food and Drug Administration for the

treatment of depression since 2008 and is clinically available in North America (134). Neuroimaging data suggest that treatment response from rTMS is associated with neural changes in frontal and limbic regions associated with MDD (135, 136), and that this varies according to the stimulation parameters chosen (137, 138). Clinical rTMS studies have shown efficacy in the reduction of depressive symptoms following a typical intervention of two weeks of daily sessions (139, 140). However, TMS comes with some (low) risk of seizure in patients with epilepsy (141), and the specialised equipment increases the cost of treatment and limits the scope for this intervention clinically, unlike other simpler methods of brain stimulation, such as transcranial direct current stimulation.

1.3.3. Transcranial Direct Current Stimulation

1.3.3.1. Technique

Transcranial direct current stimulation (tDCS) is a neuromodulatory technique that uses weak electrical current to modify the probability of spontaneous neural activity in the stimulated brain region, by acutely increasing or decreasing resting membrane potential (142). Induced changes in tissue excitability can persist over minutes to hours after stimulation, are NMDA receptor-dependant, and are presumed to reflect changes in synaptic efficacy and plasticity (143-146). Battery powered stimulation devices (see Fig. 1.3.3.1.1) are simple to use and inexpensive, with the development of home use devices broadening the potential for clinical research.

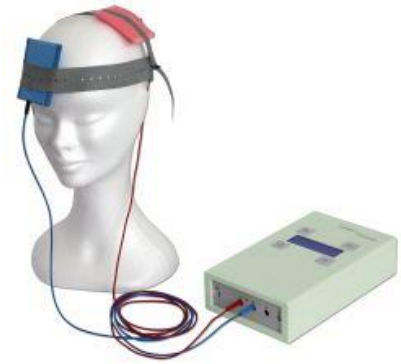

Figure 1.3.3.1.1. Battery generated unit (147) with a two cables (red anode and blue cathode). These are inserted into rubber electrodes which are encased in saline soaked sponges and affixed to the scalp using a rubber band.

In human experiments, the primary motor cortex is the most commonly targeted brain region to characterise the physiological effects of tDCS, because of the ability to measure induced excitability changes using motor-evoked potentials (MEPs). Stimulation of the human motor cortex with anodal tDCS increases MEP amplitudes[1], with the after-effects of 13 minutes of stimulation still measurable up to 90 minutes after a single session (148). A neuroimaging measure using magnetic resonance spectroscopy (MRS) indicates that anodal tDCS may influence cortical excitation through locally reduced concentrations of inhibitory neurochemical GABA, whilst cathodal stimulation may influence cortical inhibition though reduced concentrations of excitatory neurochemical glutamate[2] (149). Separate to the acute resting membrane potential changes, the post-stimulation effects of tDCS are thought to be NMDA receptor dependant, reflecting changes in synaptic efficacy. This is demonstrated by the ability of NMDA receptor agonists/ antagonists to selectively prolong/ block the post-stimulation MEP effects of tDCS (143-145). There is also evidence suggesting even longer lasting effects of repeated sessions of tDCS, with sustained MEP after-effects over 24 hours after stimulation (146); an effect which is also

[1] MEPs elicited by Transcranial Magnetic Stimulation (TMS) of the motor cortex.
[2] Cathodal stimulation also resulted in correlated (smaller) reductions in GABA, though the overall neurochemical effect is inhibitory.

blocked by an NMDA receptor antagonist. This psychopharmacological evidence suggests a role for NMDA receptors (and hence glutamate) in the after-effects of tDCS. The role of glutamate in synaptic transmission and learning/ plasticity is well established (110). Behavioural evidence implies positive effects of tDCS on motor learning (150) and even stroke recovery (151). Together with the physiological/neurochemical evidence, this indicates the potential for tDCS to affect neuroplastic learning mechanisms such as LTP and its opposing phenomenon, long-term depression (LTD). This potential could be exploited to redress pathology in clinical conditions which exhibit maladaptive neuroplasticity.

1.3.3.2. Treatment of MDD

Many neural disorders involve changes in the excitability balance within neural circuits. This makes tDCS of interest clinically as it can be used to alter the balance of excitation and inhibition in relatively focal brain regions (152), and potentially remediate aberrant excitability and maladaptive plasticity. The difficulty in treating mood and anxiety disorders (17) has brought about a renewed clinical interest in alternative treatments, such as tDCS of the DLPFC (153). Numerous studies published in the last 5 years indicate that repeated administration of pre-frontal tDCS is a potential effective treatment for depression (154-156). These studies are summarised in section 1.3.3.3., a parameter driven literature review. A recent meta-analysis (Brunoni, personal communication ahead of publication) of individual patient data from 289 tDCS treated patients shows a similar number needed to treat (7 for response, 9 for remission) as antidepressant drugs. One recent clinical trial in a sample of 120 depressed patients (156) compared repeated treatment with DLPFC tDCS with treatment using the antidepressant drug sertraline (50 mg/day). The results suggested that the combined effects of tDCS and sertraline relieved

depressive symptoms more quickly and to a greater extent than either treatment alone. In addition, tDCS showed a similar level of efficacy to sertraline, but only tDCS (and not sertraline) was superior to placebo in this trial. These results highlight the potential value of tDCS in the treatment of mood disorders. However, there is much heterogeneity in the methods used in clinical trials of tDCS and the ideal parameters have not yet been established. Therefore an experimental medicine model is needed to optimise this intervention. This will also allow an exploration of potential mechanisms of action of tDCS, compared to more traditional antidepressant treatments.

1.3.3.3. Parameter driven literature review

When setting up a tDCS study, there are many factors to consider in terms of the stimulation parameters. An extensive literature review was completed before any initial experimental design was finalised, examining research to date on non-invasive brain stimulation in the treatment of mood disorders. Twenty-eight separate tDCS studies were reviewed with regard to the specific protocol used, and were summarised and cross checked against two separate meta-analyses/reviews (153, 157) to ensure the information set was as complete as possible. This allowed parameters for the first, healthy volunteers study (Chapter Two) to be established, based on previous successful studies (summarised in Appendix I) and communications with the authors of previous studies.

In terms of stimulation region, the left dorsolateral pre-frontal cortex (DLPFC) was chosen as the anodal stimulation site for nearly all of the most recent studies (for a review see (153)). As this is a region implicated in frontal regulation of the limbic system it therefore could have implications for anxiety disorders and depression. A left/right imbalance in DLPFC activity is implicated in stress (84). Positron emission tomography

(PET) resting-state studies reveal reduced cerebral blood flow and metabolism in the left DLPFC and increased metabolism in the right DLPFC in acute MDD (158, 159). RTMS protocols also indicate that depressed patients benefit from high-frequency (fast, i.e., increasing cortical activity) rTMS over the left DLPFC and low-frequency (slow, i.e., suppressing cortical activity) rTMS over the right DLPFC(for a review see (160)). Furthermore, neuroimaging in depressed patients reveals hypoactivity in the left DLPFC and hyperactivity in the right DLPFC during emotional judgment, with hyperactivity in the right DLPFC correlating with depression severity in this group (161).

Two different montages of electrode placement are most commonly used in the clinical depression literature. Both of these montages are bipolar, placing two electrodes on the head, rather than having an extra-cephalic reference electrode. Both montages place the anodal electrode over the left DLPFC (F3 in the 10/20 system of electrode placement), but differ in where the cathodal electrode is placed. In line with recommended tDCS montage naming conventions (162), these two montages are referred to as 'bipolar-balanced' (156, 163), in which the cathode is placed over the right DLPFC, and 'bipolar-unbalanced' (154) (155), in which the cathode is placed over the right supraorbital ridge, a frequently used reference location in tDCS studies (e.g. of the motor cortex) to minimise local cortical excitation (164). Effect sizes in the treatment of depression vary across studies and there is no consistent pattern of efficacy as a function of electrode montage, though this is difficult to assess fully, given multiple differences in procedures across studies (e.g. current strength and number of sessions). Correspondence with the authors of previous papers confirmed that this was not a straightforward choice and it appeared that there was no consensus among the scientific community on this. Therefore it was decided to have two active conditions in the first experiment, as well as sham stimulation, so that

the differences between these two montages (if any) might be directly compared. This is one advantage of using an experimental medicine model to refine parameters for future studies.

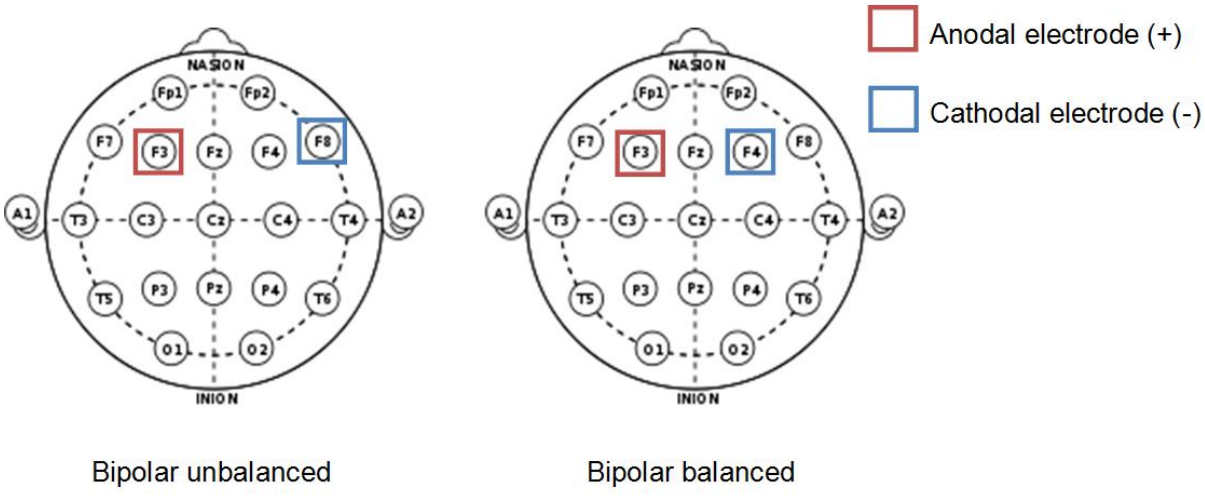

Figure 1.3.3.3.1. Two active electrode placement montages selected for first study using EEG 10/20 coordinates (F3= Left DLPFC; F4= Right DLPFC; F8= Supraorbital ridge)

The next aspect of the design to be decided was stimulation current strength, which varied between 1mA and 2mA in the literature. Of particular interest were two recent studies from the same group (155, 165) with similar experimental design, yet differing in current strength and number of sessions: 2mA for 15 sessions produced statistically significant changes in MADRS (depression) scores, whereas 1mA for 10 sessions did not produce a significant change. The number of sessions is a potential confounding factor in comparing these two studies directly. However, a higher proportion of recent successful studies had used 2mA and this appeared to be an emerging consensus. Therefore 2mA was chosen as the initial current strength.

From the perspective of emotional processing, early/acute effects of antidepressant treatment are especially of interest, given that in previous drug studies (166, 167) this was

predictive of later clinical improvement. These effects have also been shown in healthy subjects after a single dose of an antidepressant drug (167). These early effects have not been extensively explored in tDCS research, with most prior studies involving a minimum of ten sessions of stimulation and conventional clinical measures such as standardised mood questionnaires. A single session of tDCS (1mA, 10 min, bipolar unbalanced DLPFC) in healthy participants, results in an improvement in reaction times to positive-emotional versus non-emotional faces (168). Another study with a depressed group shows that a single session of tDCS (2mA, 20 min, bipolar balanced DLPFC) inverts an attentional bias to negative words in an emotional Stroop task (169). A comparison of the effects of a single session of tDCS (2mA, 30 min, bipolar balanced DLPFC) on emotional and non-emotional working memory in healthy and depressed subjects (170), reveals a specific improvement in emotional working memory in patients and not healthy controls, indicating a potential mechanism of action for its antidepressant effects. Although promising, these studies do not use tasks previously validated for prediction of psychiatric treatment response and therefore these results do not directly indicate potential treatment efficacy.

The initial design parameters, based on a literature review at Oct 2012, were optimised as the programme of work progressed.

1.4. Aims of this thesis

The hypothesis which this thesis aims to test is that tDCS relieves the symptoms of mood and anxiety disorders through an alteration of emotional bias.

In order to investigate tDCS as a potential treatment for mood and anxiety disorders, it should be compared to common clinical interventions such as antidepressant drugs. This is the first time that the cognitive neuropsychological model of antidepressant drug treatment has been applied to a non-invasive brain stimulation intervention. Behavioural tasks previously validated for antidepressant and anxiolytic effects are used in this programme of work, in order to allow direct comparisons to be made and parameters to be refined

To understand potential mechanisms of action of tDCS as a treatment for depression, the neural features of antidepressant and anxiolytic treatment should be considered. Previous tDCS studies of depression (see Appendix I for a summary) have focused on delivering tDCS to the left DLPFC. This is an area implicated in dysfunctional network integration in depressed patients, in terms of disrupted top-down control of the amygdala by the PFC. This is shown in fMRI studies on abnormal responses to negative feedback (171) and emotional/ non-emotional information processing (119). This aberrant pattern of activation is also a feature of high trait anxious healthy individuals when processing threat related stimuli (123). Given the neural features of mood and anxiety disorders, tDCS has the potential to augment pre-frontal function and improve the top-down control aspect of emotional processing. Imaging tasks which probe the emotional cortico-limbic circuit are used in this programme of work in order to detect an effect of tDCS at a neural level.

This thesis seeks to combine non-invasive brain stimulation techniques with the cognitive neuropsychological model of mood and anxiety disorders and carry out a series of experiments to explore the effects of tDCS on emotional processing, mood and

trait/state anxiety, first in healthy people and subsequently in patient groups. Study One (Chapter Two) describes an initial, healthy volunteer study of the effects of tDCS on emotional processing, where it was found that stimulation with a bilateral balance frontal electrode montage reduced vigilance to threatening faces. This helped refine the stimulation parameters for further studies. Study Two (Chapter Three) continues on from this and explores the effects of tDCS as an adjunct to attentional bias modification training, of which the results did not reveal an effect. Study Two also encompassed a biological measure of HPA axis activity (cortisol awakening response), which was reduced by active tDCS (Chapter Four). Study Three (Chapter Five) attempted to further understand the null results from Study Two, with a subsequent investigation of tDCS and ABM in anxious participants, carried out as part of a research visit to the University of Western Australia. However, the results of this study also did not reveal an effect. The repeated use of certain stimulation parameters, questionnaires and tasks allowed a pooled analysis to be carried out which enabled greater statistical power than earlier studies and also allowed additional comparisons to be made between training and non-training groups (Chapter Six). Finally, an imaging investigation (Study Four, Chapter Seven) in anxious females revealed a potential neural mechanism of the reduced vigilance to threat found behaviourally in Study One, with a proposed causal relationship between the frontal cortex and the amygdala in fear processing. The ultimate aim of this body of work is to understand and improve how tDCS might be applied in the treatment of mood and anxiety disorders and the general discussion outlines how this research can be taken forward.

Chapter Two

Frontal cortex stimulation reduces vigilance to threat: Implications for the treatment of depression and anxiety disorders

2.1. Introduction

The difficulty in treating mood and anxiety disorders (17) has raised clinical interest in alternative treatments, such as transcranial direct current stimulation (tDCS) of the dorsolateral prefrontal cortex (DLPFC) (153). A number of recent studies have indicated that repeated administration of prefrontal tDCS may be an effective treatment for depression. However, the optimal parameters for stimulation in the treatment of depression have not been established and there is still uncertainty about the underlying mechanisms of action. Following the parameter driven literature review the current study therefore directly assessed the effects of each of the two most common DLPFC stimulation montages (bipolar-balanced and bipolar-unbalanced) in changing emotional processing compared to normal (sham tDCS condition), using an experimental medicine model relevant to depression and anxiety disorders.

The cognitive neuropsychological model of antidepressant drug action suggests that treatments for depression (and anxiety disorders) work by reversing negative biases (172) and that these changes in emotional processing can be observed acutely in healthy people (112) and clinical groups (166), in the absence of acute mood improvements. Acute behavioural measurements can be designed to selectively probe distinct domains of

altered cognition characteristic of different disorders, such as vigilance to threat observed at short stimulus durations in anxiety disorders (115) versus more effortful interpretive cognition at longer stimulus durations known to be affected by depressive symptoms (173). This is because different stimulus characteristics are relevant to the cognitive schemata that underlie distinct (albeit partially overlapping) disorders (174), such as threat in anxiety disorders versus sadness in depression (175). Dot probe studies in distinct clinical groups, in which short and long duration trials have been interleaved within a single test session, have demonstrated this dissociation of negative biases at short durations in anxiety disorders versus at longer stimulus durations in depression (176, 177). Hence, to test whether tDCS would induce more of an anxiolytic-like versus antidepressant type effect, a similar measurement approach was used in the present study. Hence, acute positive shifts in emotional processing induced by an intervention (e.g. tDCS) may provide a cognitive biomarker for understanding and screening different treatments for depression and anxiety disorders, and allow optimal dosage parameters to be determined prior to a full scale randomised controlled trial.

Depression has been associated with over-activity of limbic areas, such as the amygdala, in response to negative stimuli, coupled with deficient regulation of these responses by higher order areas including the DLPFC (117, 158). Such processes are believed to contribute to the increased salience of, and behavioural response to negative information in depression. The application of tDCS over the DLPFC in depression has been suggested to work by increasing pre-frontal regulation of limbic responses to negative stimuli (169). It was hypothesised that if tDCS increases prefrontal regulation during negative emotional processing, then this would predict reduced attention and/or memory for negative versus positive emotional material after stimulation. In addition,

attentional control is highlighted in models of trait anxiety (123) and DLPFC activity has been negatively correlated with trait anxiety in neuroimaging studies examining attentional control over emotional (85) and non-emotional (124) stimuli. This suggests that modulating DLPFC activity has the potential to causally modify attentional control which has particular relevance to trait anxiety.

Emotional processing has not been extensively examined in brain stimulation research. TMS over the medial frontal cortex is shown to impair the processing of angry facial expressions in healthy volunteers (178). A recent study in healthy volunteers indicates that attentional bias modification training (ABM), involving training attention away from threatening stimuli, can be enhanced when it is paired with tDCS (1mA, 17 min, monopolar left) to the DLPFC (179), indicating a causal role for DLPFC activity in the development of attentional control relevant to anxiety disorders. However, in the literature with clinical groups, tDCS in depression has only been applied while patients were at rest (for a review, see (153)). Therefore, to maximize relevance of the current study to the existing literature describing efficacy in these clinical groups, the acute effects of tDCS applied while participants were at rest were examined. The most comparable previous study in the literature involves a single session of tDCS (1mA, 10 min, bipolar-unbalanced DLPFC) in healthy participants, which speeds reaction times to positive and negative emotional faces (168). Another study with a depressed group shows a single session of tDCS (2mA, 20 min, bipolar-balanced DLPFC) improves cognitive control for positive relative to negative words in an emotional Stroop task compared to sham stimulation (169). Although promising, both of these studies use tasks not previously validated for prediction of psychiatric treatment response and therefore these results do not directly indicate potential treatment efficacy. The current phase I, experimental medicine

model study therefore assessed the effects of a single 20 minute session of bipolar-balanced and bipolar-unbalanced tDCS to the DLPFC compared to sham stimulation, in a sample of healthy volunteers, using a battery of cognitive measures previously validated to detect early effects of antidepressant and anxiolytic treatment in both healthy volunteers and in psychiatric patient groups.

2.2. Methods and Materials

2.2.1. Participants

Ethical approval was obtained from the University of Oxford Central University Ethics Committee (MSD-IDREC-C1-2013-03CUREC). 60 healthy participants (aged 18-45, 30 female) were recruited. Participants were recruited using print and online advertisements. They were reimbursed for their time and travel expenses at a rate of £10 per hour. Exclusion criteria included any history of a psychological disorder, any psychoactive medication in the last three months, any family history of bipolar disorder and any other contraindications to tDCS. A familial history of bipolar disorder was specifically excluded as a precaution because case studies in the literature have reported potential induction of a manic state with tDCS (156, 180, 181). An initial screening session was carried out at the Warneford hospital. After informed consent was obtained, participants were screened using the structured clinical interview for DSM-IV disorders to rule out any current or prior axis I and II conditions. In addition, participants were asked to complete the Eysenck Personality Questionnaire (EPQ) and the National Adult Reading Test (NART).

2.2.2. Design

This study used a between-groups double-blind design with three groups of 20 participants randomised to each condition (active bipolar-balanced DLPFC tDCS, active bipolar-unbalanced DLPFC tDCS, or sham tDCS - which condition was randomized across both active stimulation montages). On the day of the study participants filled out some mood questionnaires before receiving tDCS while they sat at rest. After the stimulation ended the questionnaires were repeated and then participants carried out a series of computerised emotional processing tasks.

2.2.3. Transcranial Direct Current Stimulation (tDCS)

Stimulation was delivered using a battery powered device (DC Stimulator Plus, Neuroconn, Germany (147)). The rubber electrodes (25cm²) were placed in saline soaked sponges and affixed to the scalp with a rubber band. The two active conditions differed in the electrode montage used. The bipolar-balanced active condition had the anode (positive) electrode on the left DLPFC and the cathode (negative) electrode on the right DLPFC (F3 and F4 respectively, in the 10/20 system of electrode placement). The bipolar-unbalanced active condition had the same anode placement but the cathode was placed on the supraorbital ridge (F8 in the 10/20 system). The electrode placement was carried out by measuring the distance between each participant's nasion and inion and the left and right ear canals to find the vertex and then using this location as a guide to place an EEG cap on the head which was then used to locate F3 and F4/F8, which were marked using a chino graph pencil. To reduce electrode impedance the stimulation location was cleansed using an alcohol wipe and chloride scrub before the electrodes were affixed. Stimulation (20 minutes at 2mA) was applied while the participant sat at rest. In the sham condition participants received 30 sec of direct current, followed by impedance control

with a small current pulse every 550 ms (110 μA over 15 ms) instead of the stimulation current, resulting in an instantaneous current of not more than 2 μA. This method of sham stimulation produced the physical sensations typical of active tDCS and, in addition, allowed for effective double blinding by displaying realistic impedance values on the device display (147). The experimenter was thus blind to the stimulation condition, facilitated by a 'study' mode for blinding on the device. By necessity, the experimenter was not blind to the electrode montage (bipolar-balanced versus unbalanced), but as part of the blinding procedure sham tDCS was applied using a bipolar-balanced montage in half the participants while the other half received the bipolar-unbalanced montage.

2.2.4. Mood questionnaire measures

Participants filled out baseline mood questionnaires, including the Beck Depression Inventory (BDI) (10), Positive and Negative Affective Schedules (PANAS)(182) and a Visual Analogue Scale (VAS) of emotions (Happy, Sad, Hostile, Alert, Anxious, Calm). The PANAS and VAS measures were repeated after stimulation. The questionnaires were included as a control measure, as there was no *a priori* expectation of acute mood changes resulting from tDCS.

2.2.5. Behavioural measures

The behavioural tasks were carried out in a predetermined order (Fig. 2.2.5.1).

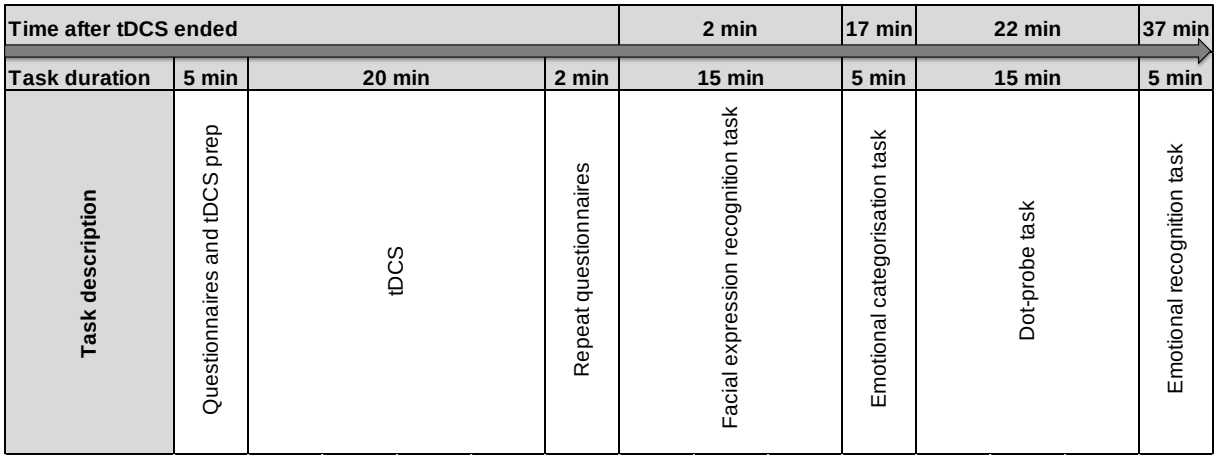

Figure 2.2.5.1. Timeline of tDCS and behavioural tasks. Values above the arrow denote time elapsed after tDCS (min), values below the arrow denote task duration (min).

Recognition of facial expression, emotional categorisation and memory were assessed as previously described (183). In brief, facial expressions associated with six emotions (anger, disgust, fear, happiness, sadness, surprise) were presented on a computer screen. Using computer graphic techniques, these expressions were averaged in 10% steps between 10% and 100% emotion (40 examples of each emotion were presented over 15 minutes). Each of the 10 actors was also presented while showing a neutral facial expression. Each stimulus remained on the screen for 500 ms, after which the volunteers responded by pressing labelled keys. This task was programmed in bespoke stimulus presentation software (University of Oxford) and was competed roughly 2 minutes after the tDCS stimulation ended.

To assess emotional categorisation, 60 personality characteristics selected to be extremely likable or unlikable (184) were presented on the computer screen for 500 ms (matched on word length, frequency, and meaningfulness), for a total task time of 5 minutes. The volunteers were asked to categorise these personality traits as likable or unlikable as quickly and as accurately as possible using a key press. This task was

programmed in bespoke stimulus presentation software (University of Oxford) and was completed 15-20 minutes after the tDCS stimulation ended.

To assess vigilance to threat, participants were presented with a series of image pairs (faces) on a computer screen and asked to respond as quickly and as accurately as possible when they saw a probe (two dots in two different orientations) appear behind one of the image pairs (192 pairs over 10-15 min) (see Fig. 2.2.5.2.). Dot probe task stimuli were fully counterbalanced for emotional face location (top vs. bottom), dot pair orientation (horizontal versus vertical) and stimulus-response button press mapping (left/right). Two different stimulus durations were used (100 or 1000 ms, in separate blocks) in order to probe distinct cognitive biases. This task was programmed in E-prime stimulus presentation software (E-prime 1.2, Psychology Software Tools, Sharpsburg, PA (185)) and was completed 20-25 minutes after the tDCS stimulation ended.

There were three different possible face stimuli pairs: happy-neutral, fearful-neutral, and neutral-neutral. The dot probe task typically uses either angry or fearful faces as the negative emotional stimulus. It has been shown, using simultaneous measures of reaction time and eye movements, that vigilance scores do not differ regardless of which face type is used (103). However, neuroimaging research typically indicates that the amygdala is more reactive to fearful than angry faces (186, 187). For this reason, much recent dot probe research has used fearful faces (188, 189), as in the present study.

'Emotional vigilance' was operationally defined as the difference in reaction time to the probe when it was located behind the emotional face (happy/fearful) of a pair versus when the probe was located behind the neutral face of the same type of pair. Thus, the

probe measures the degree of attentional allocation to the emotional faces relative to the neutral faces. Increased vigilance to threat (positive values) is indicated by faster reaction times when the probe is behind the fearful face versus the neutral face (in neutral-fearful pairs). Reduced vigilance (negative values) is indicated by slower reaction times (e.g.: when the probe is behind the happy face versus the neutral face in neutral-happy pairs). When the reaction times for emotional and non-emotional faces are the same or very similar, there is no vigilance. The faces dot probe task interleaved an equal number of blocks of long and short trials, where the faces were shown for two different durations before the probe (100ms and 1000ms). These two different durations were both tested because each duration is thought to measure different types of emotional processing (fast versus slow) relevant to different clinical disorders (anxiety disorders versus depression) (105, 190, 191).

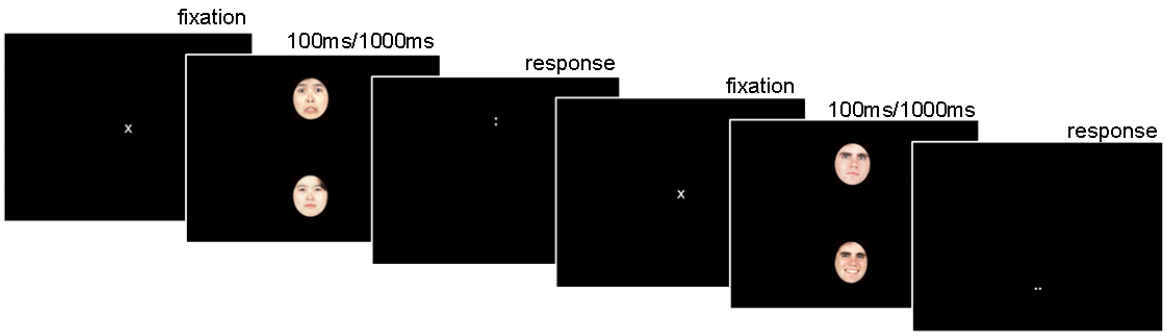

Figure 2.2.5.2. Dot probe faces task: A pair of faces precedes a probe (a pair of dots). The participant is instructed to respond to the probe with a key press.

Finally, emotional memory was examined by a surprise test of recall of the personality traits used in the emotional categorisation task, 15 minutes after task completion. The number of items recalled for each valence (minus false positives) in a two minute period was calculated. This task was completed 35-40 minutes after the tDCS stimulation ended.

Accuracy and reaction times were recorded for all tasks.

2.2.6. Calculations and Statistics

Mean accuracy scores and reaction times (RTs) were calculated for each individual for each emotion in the facial expression recognition task (neutral, anger, disgust, fear, happy, sad and surprise) and for each valence (positive and negative) in the emotional categorisation and memory tasks. Performance on the dot probe task was characterized by an index of emotional vigilance i.e. mean RT difference between the two stimulus pair types (fear vigilance= neutral - fear, happy vigilance= neutral - happy). For all tasks, RTs were calculated based only on correct trials and outliers were removed (i.e. RTs < 200ms or >1200ms, or RTs +/- 3SD from an individual participant's mean). Outlier removal resulted in < 3% of data being removed on any task. Since the goal of the study was to test whether either of the two DLPFC stimulation montages changed emotional processing compared to normal, the data from each of the tasks were analysed using analysis of variance (ANOVA), with tDCS condition (bipolar-balanced, bipolar-unbalanced, sham) as the between-subjects variable and the relevant task measures as within-subject variables. Significant main effects or interactions involving tDCS were further investigated using planned contrasts of each of the two active tDCS conditions against sham. Multiple comparisons were corrected for within-tasks but not between-tasks. Sphericity violations were corrected using the Huynh-Feldt procedure. The experimenter remained blinded until the main analysis had been completed. Analyses were conducted using IBM SPSS Statistics 20 (192).

2.3. Results

2.3.1. Group matching

The groups did not differ in terms of gender, age, highest education level, personality profile and baseline mood questionnaire measures. Baseline sub-clinical levels of depression (measured with the BDI) did not differ between the groups $F(2, 57)= .235$, $p=.792$. Baseline mood scores on the PANAS and VAS also did not differ between the groups (PANAS: $F(2, 52)= 1.453$, $p=.243$, VAS: $F(6,150)= .677$, $p=.660$).

2.3.2. Questionnaire based measurements

PANAS change: Change scores (post – pre) were analysed using rm-ANOVA with tDCS (sham, active bipolar-unbalanced, active bipolar-balanced) as a between-subjects variable and valence (positive, negative) as a within-subject variable. There were no statistically significant main effects of tDCS $F(2, 56)= .480$, $p=.621$ or valence $F(1, 56)= .233$, $p=.632$ and the two-way tDCS × valence interaction was also not significant $F(2, 56)= .377$, $p=.688$ (see Table 1). Therefore, as expected, stimulation did not significantly alter the PANAS affect measure.

VAS change: An rm-ANOVA was carried out on the VAS emotion change scores with tDCS (sham, active bipolar-unbalanced, active bipolar-balanced) as a between-subjects variable and emotion (happy, sad, hostile, alert, anxious, calm) as a within-subject variable. There was a statistically significant main effect of tDCS $F(2,56)= 3.933$, $p=.025$, $\eta_p^2=.123$, with the active tDCS groups showing a net reduction in VAS scores across all emotions (positive and negative) compared to the sham group, who showed a net increase in VAS scores across all emotions. There was also a statistically significant effect of

emotion F(2.8, 156.4)= 3.073, p=.033 η_p^2=.052, with overall reductions in anxiety and increases in calmness and happiness across all three groups. However, there was no significant two-way tDCS × emotion interaction F(5.6, 156.4)= 0.500, p=.795 and therefore, as expected, stimulation did not significantly alter specific VAS emotion change scores.

2.3.3. Behavioural measurements

Facial Expression Recognition Task: Rm-ANOVAs were carried out on percent correct, reaction times and misclassifications, with tDCS (sham, active bipolar-unbalanced, active bipolar-balanced) as a between-subjects variable and emotion (neutral, anger, disgust, fear, happiness, sadness, surprise) as a within-subject variable. There were no statistically significant main effects of tDCS on percent correct F(2, 56)= .119, p= .888, reaction times F(2, 56)= 1.756, p= .182, or misclassifications F(2, 56)= .183, p= .833. There were significant main effects of emotion, on percent correct F(6, 336)= 117.432, p< .001, η_p^2= .677, reaction times F(5, 278.2)= 10.031, p< .001, η_p^2= .152 and misclassifications F(1.92, 107.7)= 216.732, p< .001, η_p^2= .795, reflecting higher accuracy, faster reaction times and lower misclassifications for happy faces compared to other emotions across all stimulation conditions. However, there were no significant two-way tDCS × emotion interactions, not for percent correct F(10,290)= .314, p= .979, reaction times F(9,245)= .870, p= .550 or misclassifications F(4,101)= .583, p= .658. Therefore, tDCS did not significantly alter facial expression recognition.

Emotional Categorisation/ Memory Task: Rm-ANOVAs were carried out on reaction time and recall data with tDCS (sham, active bipolar-unbalanced, active bipolar-balanced) as a between-subjects variable and valence (positive, negative) as a within-subject

variable. There were no statistically significant main effects of tDCS on reaction times $F(2, 55)= .939$, $p= .397$ and recall $F(2, 55)= .617$, $p= .543$. There were significant main effects of valence on reaction times $F(1,55)= 31.160$, $p< .001$, $\eta_p^2= .362$ and recall $F(1,55)= 51.938$, $p< .001$, $\eta_p^2= .486$, reflecting faster reaction times and greater recall of positive items compared to negative items across all stimulation conditions. However, there were no significant two-way tDCS × valence interactions for reaction times $F(2, 55)= .132$, $p= .877$ or recall $F(2, 55)= .536$, $p= .588$. Therefore, tDCS did not significantly alter emotional categorisation or memory.

Faces Dot probe Tasks: Face pairs were presented at two different durations, short (100ms) and long (1000ms), which were analysed independently. Analysis of short-duration trials aimed to test whether tDCS would abolish fear vigilance that is characteristic of rapid responses to threat seen in anxiety disorders. Analysis of long-duration trials tested whether tDCS would abolish fear vigilance on a slower timescale that is characteristic of the more effortful, negative interpretative cognition seen in depression.

Short duration dot probe task (100ms): Before testing for a change in behaviour with tDCS, it was first examined whether the expected fear vigilance phenomenon was present at baseline. Paired samples t-tests were carried out on the reaction times in the sham condition, for fearful/neutral face pairs. Participants exhibited the expected pattern of significant fear vigilance (faster reaction times when the probe was located behind the fearful face versus the neutral face of a pair) $t(18)= 3.569$, $p= .002$; mean paired differences= 27.47, SD= 33.55 (Fig. 2.3.3.1.).

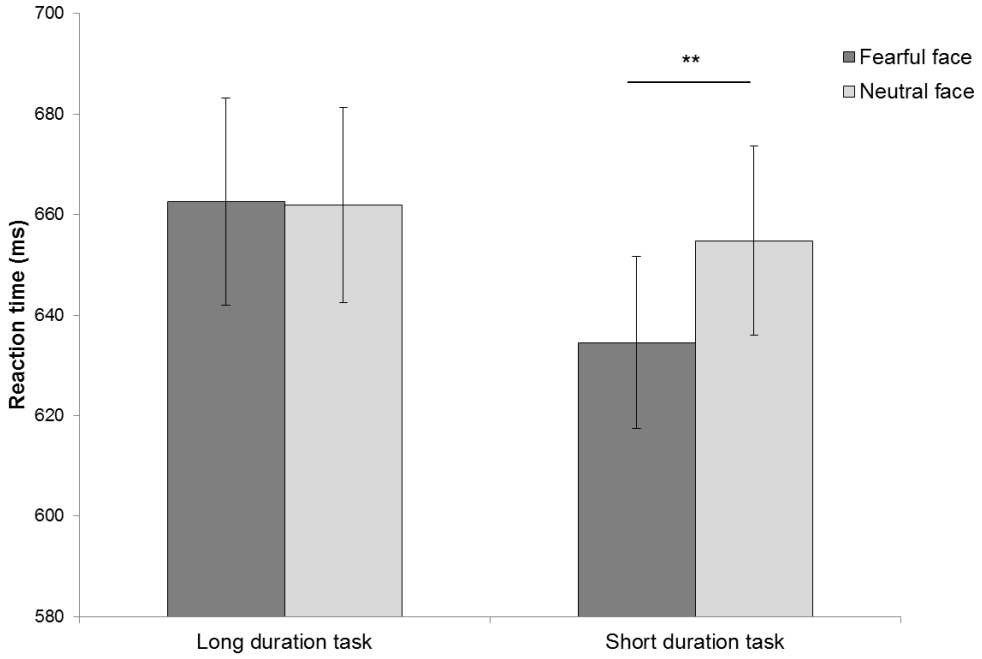

Figure 2.3.3.1. Baseline dot probe vigilance: Significant fear vigilance was present in the short duration dot probe task (100ms) but not in the long duration dot probe task (1000ms). Mean reaction times (ms) when the probe was located behind the fearful face of a fearful/neutral pair versus when the probe was located behind the neutral face of a fearful/neutral pair. Asterisks (**) denote a significant decrease in reaction times for fearful faces compared to neutral faces (p= .002). Error bars represent +- 1 standard error of the mean.

To assess the effects of tDCS on the baseline (sham) pattern of emotional vigilance, rm-ANOVA was carried out on the vigilance scores with tDCS (sham, active bipolar-unbalanced, active bipolar-balanced) as a between-subjects variable, and emotion (happy, fearful) as a within-subject variable. As expected, there was a significant main effect of emotion $F(1,54)= 7.494$, $p= .008$, $\eta_p^2= .122$ reflecting overall positive vigilance scores for fearful faces ($M= 10.65$, $SD= 38.95$) and negative vigilance scores for happy faces ($M= -9.46$, $SD= 34.822$). Although the main effect of tDCS was not significant $F(2,54)= .857$, $p= .430$, there was a significant two-way emotion × tDCS interaction $F(2,54)= 3.251$, $p= .046$, $\eta_p^2= .107$, suggesting that the baseline pattern of emotional vigilance was altered significantly, in a manner that depended on the tDCS configuration.

To clarify the nature of the tDCS-induced change in emotional vigilance, two separate one-way ANOVAs were conducted on vigilance scores for each emotion. For vigilance to happy faces there was no effect of tDCS $F(2,54)= .890$, $p= .417$, but for vigilance to fearful faces there was a significant effect of tDCS $F(2,54)= 3.594$, $p= .034$, $\eta^2= .116$. Planned contrasts against the sham condition revealed that this significant between-groups effect was driven by a significant reduction in fear vigilance in the active bipolar-balanced tDCS condition compared to sham $t(54)= 2.679$, $p= .005$; active bipolar-balanced: $M= -4.55$, $SD= 39.81$; sham: $M= 27.42$, $SD= 33.65$. Whereas participants in the sham tDCS condition exhibited the expected pattern of greater vigilance towards fearful and reduced vigilance towards happy faces, this was abolished by active bipolar-balanced tDCS (Fig. 2.3.3.2.). 1-sample t-tests (2-tailed, against zero) on the vigilance scores confirmed that participants in the active bipolar-balanced condition neither showed significantly greater vigilance towards fearful faces $t(19)= -.511$, $p= .616$, nor significantly less vigilance towards happy faces $t(19)= -.153$, $p= .880$, by contrast with the sham condition (fearful faces: $t(18)= 3.552$, $p= .002$, happy faces: $t(18)= -1.960$, $p= .066$). Hence, participants in the bipolar-balanced tDCS condition showed similar vigilance towards fearful, happy and neutral face stimuli. Although the active bipolar-unbalanced tDCS condition showed a similar numerical trend (reduction in fear vigilance), this did not reach statistical significance $t(54)= -1.435$, $p= .158$. Direct contrast of the two active tDCS conditions was also not significant $t(54)= 1.188$, $p= .480$, and the observed pattern of data (intermediate between the sham and bipolar-balanced conditions, (Fig. 2.3.3.2.) suggests that bipolar-unbalanced tDCS had only a partial attenuating effect. Hence, only bipolar-balanced tDCS significantly abolished the normal pattern of fear vigilance. A one-way ANOVA revealed no effect of tDCS on accuracy in this task $F(2,52)=.818$, $p=.447$.

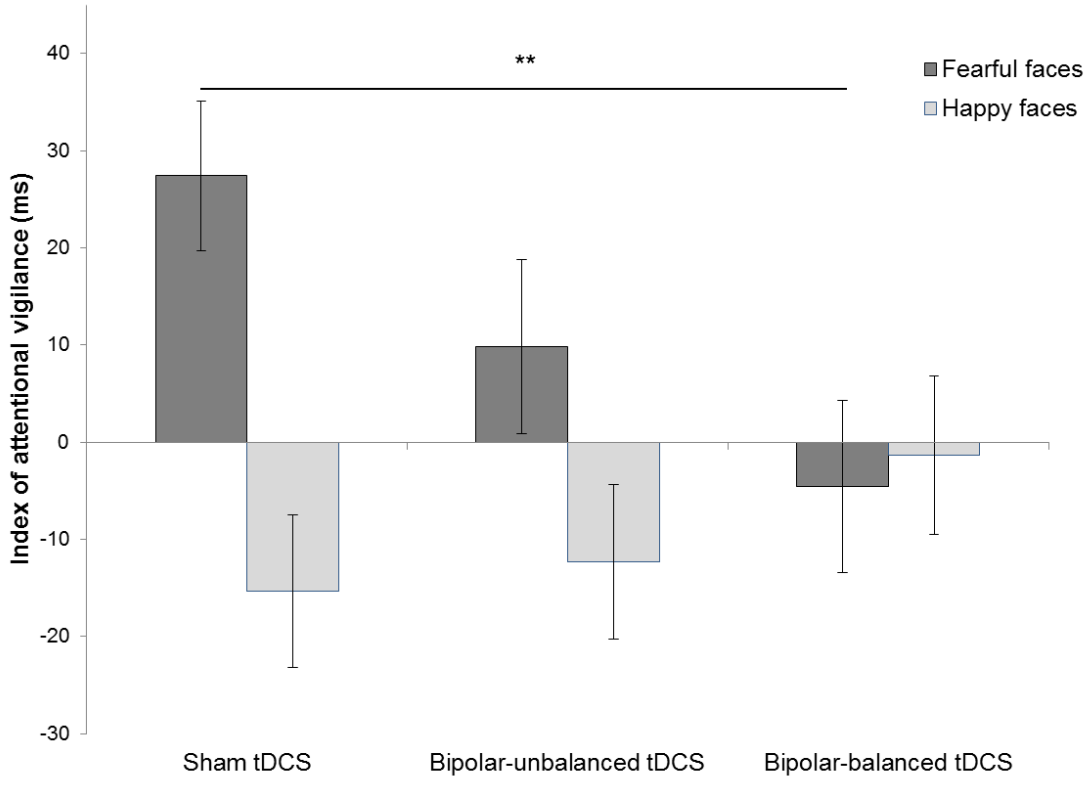

Figure 2.3.3.2. Dot probe emotional vigilance. Vigilance scores represent the difference between reaction times (ms) when the probe was located behind the emotional face versus when the probe was located behind the neutral face of the same pair type. In the sham condition, participants exhibited the expected normal pattern of increased vigilance to threat (positive values on y axis), and reduced vigilance to happy faces (negative values on y axis). Bipolar-unbalanced stimulation of prefrontal cortex did not significantly alter this pattern, but bipolar-balanced stimulation abolished it. Asterisks (**) denote a significant decrease in fear vigilance in the bipolar-balanced tDCS condition compared to sham stimulation (p= .005). Error bars represent +- 1 standard error of the mean.

Long duration dot probe task (1000ms): Before testing for a change in behaviour with tDCS, it was first examined whether the expected fear vigilance phenomenon was present at baseline. Paired samples t-tests were carried out on the reaction times in the sham condition for fearful/neutral face pairs. There was no evidence of fear vigilance t(18)= -.759, p= .458; mean paired differences= 7.95, SD= 45.63 (Fig. 2.3.3.1.). Given the absence of measurable fear vigilance at baseline, there was no behavioural phenomenon present for tDCS to change. Hence, the long duration trials were not analysed further for effects of tDCS. A one-way ANOVA revealed no effect of tDCS on accuracy in this task F(2,52)= 2.221, p= .119.

2.3.4. Tolerability and side effects

In general, tDCS was well tolerated, with all participants reporting a mild transient itching/stinging under the electrodes which faded after the first minute of stimulation. One participant requested that the stimulation be terminated after 5-10 seconds of because of discomfort from the itching/stinging sensation under the electrodes. After the device was switched off, there was no lasting discomfort from this participant. No other adverse effects were reported.

2.4. Discussion

These findings provide the first experimental evidence that modulating activity in the DLPFC leads to reduced vigilance to threatening stimuli. This study is also the first to directly compare the two most common electrode montages used for DLPFC tDCS, with the results favouring the bipolar-balanced montage. In this study, the group receiving sham stimulation displayed the expected attentional bias towards fearful faces on short stimulus duration trials. Healthy participants typically show this pattern of increased vigilance (faster reaction times) for fearful faces compared to neutral faces, an effect known to be attenuated by antidepressant and anxiolytic treatment (193). This negative bias (i.e. increased vigilance to threat) was reversed with DLPFC tDCS. One of the key aims of this study was to test two DLPFC stimulation electrode montages commonly used in clinical research. The bipolar-balanced montage significantly abolished the normal pattern of fear vigilance observed in the sham condition. By contrast, although a similar numerical trend was observed in the bipolar-unbalanced condition (Fig. 2.3.3.2.), this did not reach significance. This suggests a partial effect, likely reflecting the fact that both

electrode montages involve anodal stimulation of left DLPFC. The fact that only the bipolar-balanced condition significantly changed fear vigilance from normal (sham) suggests that intervening bilaterally, to change activity in both left and right DLPFC, may be critical for the anxiolytic-like effect observed. These results highlight a role for stimulation of the DLPFC in modulating attentional bias to threat.

Attentional bias to threat, particularly at short exposure durations, has been particularly associated with anxiety disorders (194). Indeed, volunteers high in trait anxiety or with DSM anxiety disorders typically show enhanced vigilance to threatening face or word cues at short durations (195). Consistent with this, anxiolytic interventions reduce bias towards threat in this paradigm, an effect also seen in healthy volunteers. In particular, the administration of the SSRI citalopram (193) and the benzodiazepine diazepam (114) were found to reduce fear vigilance in healthy volunteers using a similar dot probe task. A parallel study using an exposure-based cognitive behavioural intervention for panic disorder also revealed an early effect of treatment on vigilance to fearful faces and this effect predicted subsequent reduction in panic disorder symptoms following treatment (115). Together, these results suggest that tDCS has an anxiolytic-like profile in this task, and that these effects can be seen most clearly with bipolar-balanced DLPFC stimulation. Since a similar pattern of effects on this task has been observed previously both in studies examining the effects of anxiolytic drugs on healthy volunteers and patients undergoing anxiolytic treatment, it is possible that the effects shown in the present study reveal a common mechanism of action for previously reported effects of tDCS on standard measures of mood in clinical trials (156).

TDCS did not lead to a widespread change in emotional processing across the other measures used in this study. Whereas the other emotional processing tasks in the present study (such as facial expression recognition, emotional categorisation and emotional memory) are typically used to assess antidepressant treatment (173, 196), the dot probe task is mainly used to measure anxiolytic treatment (114, 197). The present study suggests that bipolar-balanced DLPFC tDCS reduces vigilance to threat-related emotional stimuli, reminiscent of specific effects seen with anxiolytics like diazepam in this task (114). Depression and anxiety disorders are the most highly comorbid psychological disorders in primary health care (198) and it can be difficult to separate them in terms of diagnosis and treatment. There is a possibility that the antidepressant effects of tDCS observed in the literature are related to anxiolytic effects. This is supported by a study which found that during negative imagery visualization, anodal tDCS led to decreased cortisol levels and increased vagal activity, measures which reflect stress regulation (199). Cortisol awakening response is a useful proxy of depression and trait/state anxiety or stress and so future investigations could include this measure to add to these behavioural results.

The present phase I study in healthy volunteers identified an anxiolytic-like profile of tDCS that was specific to the active bipolar-balanced DLPFC stimulation condition. The next logical step is to assess whether the effects reported here in a healthy volunteer experimental medicine model can be found in a trait anxious group or a patient group, and if they can predict response to treatment of depression and anxiety disorders. The results from this study support the use of bipolar-balanced DLPFC stimulation paradigms and measurements of attentional bias. These measurements have been identified as a marker of efficacy in other treatments and so this protocol could have a similar effect in a clinical population, where reductions in negative biases have been shown to predict later clinical

improvement (115, 166). The focus of this study was to investigate acute effects of tDCS, by testing for an induced change in emotional processing similar to what was previously observed acutely with antidepressant or anxiolytic treatment. However, it will also be necessary to evaluate whether these effects seen acutely can be extended over time with repeated interventions, as occurs with antidepressant drug treatment. In addition, it could be useful to examine the efficacy of frontal cortex tDCS as an adjunct to other interventions which target attentional biases, such as attentional bias modification training or exposure therapy.

The present study demonstrates that modulating DLPFC activity can change attentional bias (fear vigilance at short stimulus durations) that is relevant to clinical anxiety disorders (200). Brain imaging studies have revealed hypo-activation of the DLPFC in trait anxiety (85, 123, 124), which is thought to reflect deficient attentional control in anxiety disorders (125). The present study suggests a causal relationship between DLPFC stimulation and attention to threat, since changing DLPFC activity abolished fear vigilance. Future studies, by combining tDCS with fMRI, could cast light on the functional brain changes that mediate this effect. For instance, reduced DLPFC activity is thought to cause reduced top-down inhibitory control over limbic regions, which are hyper-activated in response to negative emotional stimuli in anxiety disorders (117). This will be examined in a subsequent imaging investigation (Chapter Seven). In this study, emotional bias was not evident at the long stimulus duration in the dot probe task. However, further studies exploring the cognitive sub-components of vigilance to threat at different durations (e.g. difficulty to disengage, see (104)) could provide additional insights into the specific nature of the tDCS effect.

Since the dot probe task was conducted ~22 minutes after the end of stimulation, it is conceivable that, in some individuals, the physiological after-effect of tDCS may have already decayed by that time. The duration of after-effects of frontal tDCS has not been studied systematically. However, in motor cortex, the same stimulation protocol used here (2mA, 20 minutes) had effects lasting for at least 90 minutes after stimulation offset (201). In our study, the dot probe tasks were carried out ~22-37 min after stimulation. Hence, testing was conducted reliably within the likely time window of tDCS after-effect.

The current phase I results in healthy volunteers reveal an anxiolytic-like effect of dorsolateral prefrontal cortex tDCS on a cognitive biomarker relevant to clinical anxiety disorders, and indicate a potential neurocognitive mechanism (reduced fear vigilance) that may partially mediate the reported findings of clinical efficacy of prefrontal tDCS in the literature. In this first direct comparison of two electrode montages used in the depression literature, the data indicate preferential use of a bipolar-balanced rather than bipolar-unbalanced DLPFC stimulation montage.

In summary, this study offers an experimental medicine framework and a sensitive cognitive biomarker that could be used for future testing, refining and validating the novel intervention of prefrontal tDCS to mitigate the symptoms of anxiety disorders and depression. By contrast with the more empirical approach to devising tDCS interventions that characterizes the existing literature (as reflected in the great heterogeneity of stimulation parameters), this design outlines a principled approach, and describe an anxiolytic-like stimulation profile on a laboratory test of proven clinical predictive utility. Hence, the value of this study is that it offers a principled path towards testing and benchmarking novel stimulation protocols at the development phase, either in healthy

volunteers or in clinical populations, in order to optimise treatment efficacy for depression and anxiety disorders. Before a neuroimaging investigation of the observed phenomenon is attempted, intermediate studies should build on this optimisation by examining the effect of tDCS on additional measures of stress and anxiety or as an adjunct to other treatments which specifically target fear vigilance.

Chapter Three

An exploration of the effects of frontal cortex stimulation and attentional bias modification on vigilance to threat and induced anxiety

3.1. Introduction

The results from the dot probe task in Study One suggest an effect of tDCS on vigilance to threatening information similar to that observed with anxiolytic drug treatment. To explore this further, the next study focused on behavioural tasks that measure state/trait anxiety and combined tDCS with a training regime which was predicted to be synergistic. Since the efficacy (compared to sham) of the bipolar-balanced montage was established in the previous experiment, this montage was used for further studies, reducing the need for two active stimulation groups.

Research using non-invasive brain stimulation has historically been most prominent in investigations of motor function and has been usefully paired with a motor based task/training to induce improvements in motor function post stroke (151, 202-204). As the body of tDCS research in cognition is growing, the stimulation is increasingly being paired with cognitive training (205, 206) in an attempt to maximise any effects on excitability changes and potential neuroplasticity. Investigations of tDCS as a potential treatment for mood disorders such as depression have focused on delivering tDCS whilst participants were at rest, informing the design of Study One. However, given lessons learnt from other fields, it may be beneficial to activate the DLPFC simultaneously using both a task and tDCS. For example, participants could carry out a task that engages the

DLPFC and simultaneously use tDCS to increase DLPFC excitability. Options for training tasks considered included working memory tasks (207), feedback training (208) and imagery based interventions (209). Of particular potential relevance to this investigation was a form of training that specifically targeted emotional biases - Attentional Bias Modification (ABM) training.

ABM training tasks are developed to alter attentional bias to emotional information and are being investigated as a potential treatment for depression and anxiety disorders (47). ABM training has shown potential to affect emotional processing and stress reactivity in healthy volunteers (210, 211), depressed (212) and anxious (213) patients and also in young people with aggression problems (214). This training modifies attentional biases by repeatedly directing visual attention away from aversive stimuli and towards neutral or positive stimuli. Of particular significance to our investigation, neuroimaging results have shown that the modification of attentional biases using a single session of ABM is mediated by the BOLD activity in the lateral DLPFC (211). This makes the case for stimulation of the DLPFC as a potential way to causally interact with the effects of ABM.

The most commonly used ABM task is a computerized, visual-probe bias modification procedure, similar to the dot probe task used in the Study One. In this task, a pair of stimuli (two words) are briefly presented and followed by a probe (one or two dots), which appears behind one of the stimuli. Participants will be required to press one of two buttons to indicate the number of dots in the probe. The word pairs are differently valenced: neutral and threatening. During 'avoid threat' ABM training, the probes are located behind the neutral word and thus, when completing 'avoid threat' ABM training,

participants learn to deploy their attention away from negative stimuli as they learn predict the probe location. Transfer effects of this training have been shown in distinct tasks (e.g., using face stimuli (215)), mood questionnaires, lab based stressors (210) and in measures of endocrine function such as the cortisol awakening response (CAR) (48), a commonly used proxy measure in depression and anxiety disorder research (216). Placebo ABM can be delivered by changing the location of the probe, so that it is equally often found behind the positive and negative stimuli. Furthermore, 'attend threat' ABM can be delivered by changing the location of the probe so that it is always behind the threatening stimuli, which may aid separation between ABM conditions in single session investigations.

After careful consideration of the literature on training, it was decided that attentional bias modification (ABM) training was most suitable because of its focus on emotional biases, applicability to anxiety disorders and the potential role of the DLPFC in its mechanism of action.

Previous studies have involved acute, single session ABM investigations (48, 210, 212) and longer, treatment based studies with daily sessions of ABM over the course of a week or longer (48, 213, 217). The present study tested whether tDCS can potentiate the effects of single session of ABM training in healthy volunteers. During the design of this second study, Clarke et al. (179) published a paper suggesting that DLPFC tDCS has a causal role in the the success of ABM in reducing attentional bias to threat, using similar dot probe ABM training. The present study aimed to explore a similar question but additionally include alternative measures of emotional processing and a lab based stressor to explore the transfer effects of any change in cognitive bias. In addition, the electrode

montage and current strength of the present study (informed by the parameter optimisation from Study One) differed from that used in Clarke et al., making this a novel investigation.

3.2. Methods and materials

3.2.1. Subjects

Ethical approval was obtained from the University of Oxford Central University Ethics Committee (MSD-IDREC-C1-2014-04CUREC). 80 healthy participants (40 female, aged 18-45) were recruited using print and online advertisements; they were reimbursed for their time and travel expenses at a rate of £10 per hour. Screening procedures were as per Study One (Chapter Two).

3.2.2. Design

A four arm, between-groups design was carried out. This involved 20 participants receiving active tDCS and 'avoid threat' ABM, 20 participants receiving active tDCS and 'attend threat' ABM, 20 participants receiving sham tDCS and 'avoid threat' ABM and a further 20 receiving sham tDCS and 'attend threat' ABM (see Table 3.2.2.1.). Blinding protocol was as per Study One.

Group	tDCS (Bipolar-balanced DLPFC)	ABM
Group 1	2mA, 20 mins	avoid threat
Group 2	2mA, 20 mins	attend threat
Group 3	sham, 20 mins	avoid threat
Group 4	sham, 20 mins	attend threat

Table 3.2.2.1. Factorial, four arm design of tDCS stimulation and ABM training group assignment, counterbalanced and gender matched across groups.

On the day of the study participants filled out some mood questionnaires before being prepared for tDCS. The electrodes (25cm²) were placed in saline soaked sponges and affixed to the scalp with a rubber band. The tDCS (bipolar-balanced DLPFC, 20 minutes at 2mA) was applied whilst participants completed a computerised ABM training module (tDCS and ABM type defined by group allocation). The experimenter was blind to the ABM and tDCS conditions, facilitated by a 'study' mode on the device (Neuroconn stimulator plus, Neuroconn, Ilmenau, Germany, (147) see Appendix IV). After the tDCS/ABM participants were asked to carry out a series of emotional processing tasks (described below) and fill in some of the mood/ state anxiety questionnaires again. In addition, a lab based stressor (instructed worry task) was used to measure the effects of the tDCS/ABM on induced mood/state anxiety. Finally, participants were asked to complete a simple toy fishing game.

3.2.3. ABM module

The attentional bias training procedure (Fig. 3.2.3.1), was recently used by Browning et al. (211) and replicated the method described by MacLeod et al. (210). Stimuli were generated with Presentation software (version 18.1, Neurobehavioral Systems Inc., Berkeley, CA (218)). Briefly, each ABM trial commenced with the 500-ms presentation of a fixation cross in the centre of an LCD screen, which served as a fixation cue. Immediately following termination of this display, two words were presented, one word appeared just above the location of the preceding fixation cross, whereas the other appeared just below this location (see Appendix III for a full set of word pairs). After 500 ms, the words were replaced by a probe (a single dot or two dots) in the location of one of the words. The participants were instructed to respond as quickly and accurately as they could by button press to indicate whether the probe consisted of one dot or two. The word pairs used were taken from the study by MacLeod et al. (210) and consisted of a negative word (e.g. pain) and a neutral word (e.g. laws). The position of the negative word was randomized, such that it appeared either in the upper or lower screen location with equal probability on any trial. Attentional training was achieved by controlling the position of the probes such that in the avoid threat group the probes were always in the position of the neutral word, whereas in the attend threat group, the probes were always in the location of the negative word. The training task consisted of a total of 576 trials in pseudorandom order, as well as three rest sessions. Over the course of training, participants learn to attend to the valence of stimuli that predict the location of the probe to which they have to respond; therefore, the attend threat training encourages a negative attentional bias, whereas the avoid threat training encourages a tendency to avert attention from negative stimuli.

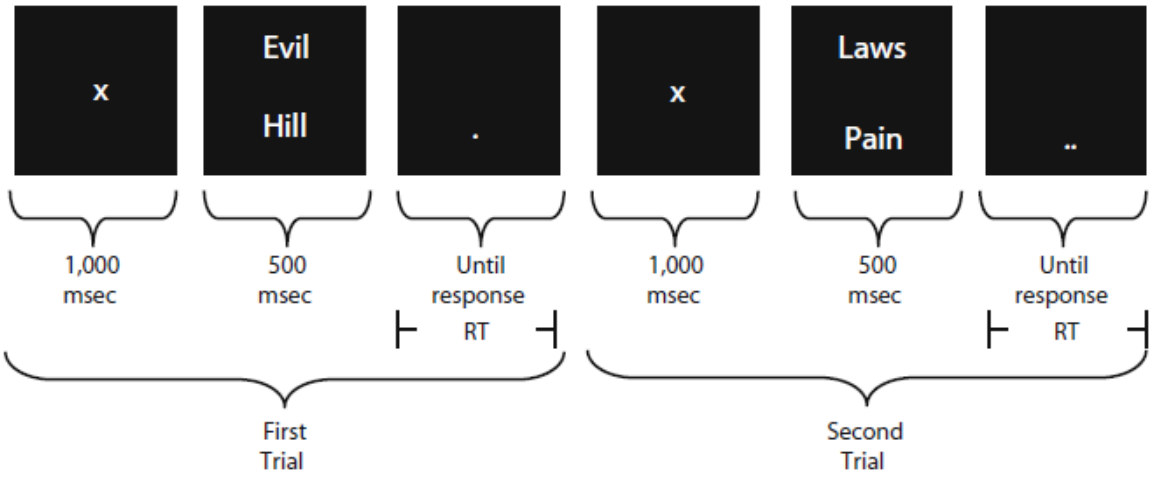

Figure 3.2.3.1. Example trials from the attentional bias training task. Two words were presented, one above the other, on a computer screen. After 500 ms, the words were replaced by a probe (a single dot or two dots) in the location of one of the words. The participants were instructed to respond by button press to indicate whether the probe consisted of one dot or two. The figure illustrates the first trial from the avoid threat training condition in which the probes always replaced the neutral word and the second trial from the attend threat training condition in which the probes always replaced the threatening words. Reproduced with permission from (211).

3.2.4. Questionnaire measures

Participants filled out baseline mood questionnaires, including the Beck Depression Inventory (BDI) (10), State-Trait Anxiety Inventory (STAI) (24), Positive and Negative Affective Schedules (PANAS) and a Visual Analogue Scale (VAS) of emotions (Happy, Sad, Hostile, Alert, Anxious, Calm). The STAI (state), PANAS and VAS measures were repeated after tDCS/ABM. The VAS measures were again repeated four times during the instructed worry task. Participants were asked to fill in online versions of all measures the day after testing, at two week follow up and at four week follow up.

3.2.5. Behavioural measurements

The behavioural tasks were carried out in a predetermined order (see Fig. 3.2.5.1.), so that the potential effects of tDCS decay could be held as constant as possible.

Time after tDCS ended				12 min	27 min	32 min	42 min	57 min
Task duration	5 min	30 min	2 min	15 min	5 min	10 min	15 min	5 min
Task description	Questionnaires and tDCS prep	tDCS & ABM (tDCS terminates after 20 minutes)	Repeat questionnaires	Faces dot-probe task	Words dot-probe task	Continuous flash suppression task	Guided worry mood induction	Fishing game

Figure 3.2.5.1 Timeline of tDCS and behavioural tasks. Values above the arrow denote time elapsed after tDCS (min), values below the arrow denote task duration (min).

Vigilance to threat was assessed using the dot probe faces task, as described in Chapter Two, with one modification. The long duration trials in the previous version of the dot probe task were replaced with medium duration trials (500ms), matching the stimulus duration of the ABM task.

Vigilance to threat was also assessed using a word based version of the dot probe task. This was almost identical in format to the ABM training module (see Fig. 3.2.3.1.). However, in the test trials the probe appeared equally often in the position of the negative word as it did in the position of the positive word. 'Emotional vigilance' is measured as the difference in reaction times to the probe when it was located behind the negative words versus when the probe was located behind the neutral words. Thus, it is a reliable measure of attention (before the probe) to the negative/neutral words.

The Continuous Flash Suppression (CFS) task used in this study was as previously described (219). Briefly, stimuli consisted of grayscale faces displaying 1 of 3 different expressions (neutral, happy, and fear) and grayscale Mondrian-like patterns as the suppressor mask. CFS stimuli were generated with the Psychtoolbox (220) for Matlab version 2010a (Mathworks, Inc., Natick, MA (221)) and presented on an LCD screen.

Stimuli were viewed using a mirror stereoscope, which was positioned in front of the monitor and connected to a chin rest. A black cardboard divider of matte material was placed between the participant's eyes, extending from the stereoscope midline toward the centre of the display to block the line of vision to the other eye's stimulus. The lights were switched off during the CFS task. Before the task was initiated, the mirrors of the stereoscope were carefully adjusted so that the fusion contours surrounding the stimuli were combined to produce a square frame, which occurred only when the eyes were appropriately aligned. In the initial 1000 ms, one eye was presented with full contrast, dynamic Mondrian patterns, while the other eye viewed a low contrast face image. The contrast of the face was ramped up at a rate of 2% every 20 ms, hence avoiding abrupt transients. When the face reached full contrast (equivalent to 1000 ms after presentation onset), the contrast of the Mondrian patterns linearly decreased at a rate of 2%/100 ms for the next 5100 ms. The face remained at full contrast until a response was made (see Fig. 3.2.5.2.). All stimuli, as well as the eye to which the Mondrian patterns or faces were presented, were randomized and counterbalanced across trials (180). The face was presented within one of the four quadrants of the square. Participants were required to report its location via one of four corresponding buttons on a keyboard, as quickly and accurately as possible. Participants were instructed to make a keypress as soon as any part of the face (such as the eyes, mouth, etc.) emerged.

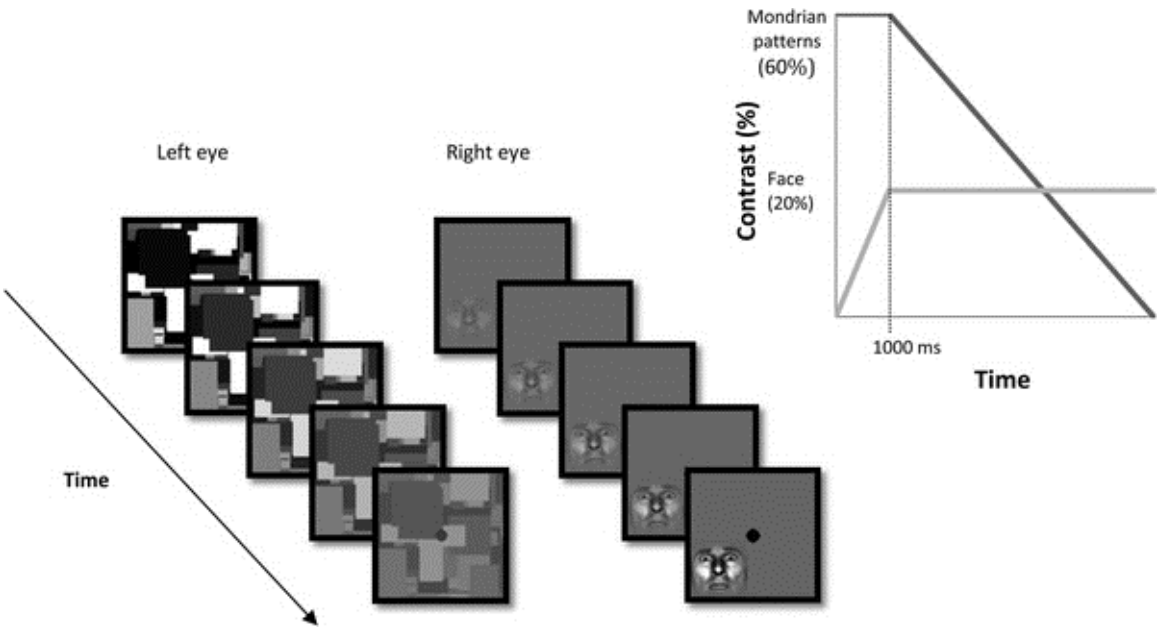

Figure 3.2.5.2. In the continuous flash suppression task participants see either a Mondrian-like pattern or face in each eye. The contrast between the two images in manipulated over the trial. Participants respond with a key-press as soon as they see any part of the face. Figure reproduced with permission with permission from (219)

Worry persistence was assessed using a computerised instructed worry task, adapted from (222). The task had three phases and was programmed in EPrime 2.0 (Psychology software tools, Sharpsburg, PA (223)). Upon confirming that instructions were understood the participants were left alone in the testing room for this task. All subsequent instructions and questions were displayed as text on a LCD screen and auditory cues were sounded through earphones. The task comprised three, five minute phases: 1) a *breathing focus* phase; 2) an *instructed worry* phase and 3) another *breathing focus* phase. At the beginning of the task, participants filled out a paper Visual Analogue Scale (VAS) rating of different emotions (happy, sad, hostile, alert, anxious, calm). Then, for the first *breathing focus* phase they were instructed (all instructions by text on the screen generated by the computer program) to try to clear their mind of all thoughts and only focus on their breathing for the next five minutes. At 12 occasions during the five minute breathing focus phase (ISI randomised between 19 and 31 sec), a tone would sound through the headphones and participants would be instructed to indicate with a key press if they were

focusing on their breathing or distracted by some other thoughts at the time of the tone. In the instances when the participant indicated that they were distracted by other thoughts they were then instructed to indicate with a key press, their emotional rating of those thoughts from negative (1) to positive (9). After the first *breathing focus* phase participants were instructed to complete the paper VAS ratings again. Next, for the *instructed worry* phase, participants were instructed to identify a topic that worried them and were instructed to silently worry about this topic for five minutes, in the way that they normally would. At the end of this *instructed worry* phase participants were instructed to complete the paper VAS ratings again. This was followed by a second *breathing focus* phase, identical to the first. Finally, after the second *breathing focus* phase was complete participants were instructed to complete the paper VAS ratings one last time. Based on previous findings (222), it was expected that participants would have higher VAS scores for sadness and anxiety and lower VAS scores for happiness and calmness, as well as a higher number of negative intrusions, following the *instructed worry* session.

Finally, participants played a fishing game, as described in (224). This simple task employed a toy measuring 910 mm × 910 mm × 250 mm, in which 8 brightly coloured plastic fish moved round in a circle, opening and closing their mouths to reveal a magnet. Participants were required to catch as many fish as they could in 2.5 min by 'hooking' them using a magnet on the end of a 900 mm plastic fishing rod. The experimenter used 8 different sets of fishing games of identical make and model.

3.2.6. Calculations and Statistics

Accuracy scores and individual means of reaction times were calculated for each emotion in the CFS task (neutral, happy, fear) or valence (threat, neutral) in the dot probe words task. The faces dot probe task required calculation of an index of emotional vigilance, as per Study One. Only correct trials were included in the reaction time calculations. Extreme outlying data was removed from all psychological tasks as per Study One. This resulted in less than 3% of data being lost as outliers on any task. For the instructed worry task, VAS change scores were calculated from the pre and post instructed worry VAS scores. There was also a count of negative intrusions in the breathing focus, pre and post instructed worry and a calculation of the mean valence of intrusions pre and post worry. All data were analysed using IBM SPSS Statistics 20 (192). Questionnaire data were analysed using two-way analyses of variance (ANOVAs) for baseline questionnaires and repeated measures (rm) -ANOVAs for repeated questionnaires, with questionnaire scores as the within-subject variable and tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables. Behavioural data from each of the tasks (including reaction times and accuracy) were analysed using rm-ANOVAs, with emotional valence of stimuli (positive, negative) as a within subjects variable and tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables. Significant interactions were followed up with planned contrasts to compare the effects of tDCS and ABM separately. Where assumptions of equality of variances were broken, a Huynh-Feldt correction was applied to degrees of freedom. Multiple comparisons were corrected for within-tasks but not between-tasks. The experimenter was not unblinded until the main analysis had been completed.

3.3. Results

3.3.1. Group matching

The groups were matched in terms of gender, age, highest education level and personality profile. Baseline sub-clinical levels of depression (using the BDI) and trait anxiety (using the STAI-T) did not differ between the groups (BDI: $F(3, 68)= .787$, $p= .505$, STAI-T: $F(3, 67)= .360$, $p= .782$).

3.3.2. Follow up data compliance

Participants were asked to complete a series of online questionnaires as part of this study. Compliance and related data loss was as follows:

Baseline data: 85% compliance

Day 2 data: 75% compliance

Day 14 data: 84% compliance

Day 28 data: 82 % compliance

3.3.3. Questionnaire based measurements (acute)

Acute PANAS change: To assess acute affective change, PANAS scores were analysed using rm-ANOVA with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and valence (positive, negative) and time (pre, post) as within-subject variables. There were statistically significant main effects of time $F(1, 70)= 23.327$, $p< .001$, $\eta_p^2= .250$, valence $F(1,70)= 558.629$, $p< .001$, $\eta_p^2= .889$ and the two-way time x valence interaction was also significant $F(1, 70)= 13.582$, $p< .001$, $\eta_p^2= .162$, reflecting higher positive scores and lower negative scores overall and a decrease in both positive and negative scores over time.

Acute VAS change: To assess acute emotion rating changes an rm-ANOVA was carried out on the VAS emotion change scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and time (pre, post) as within-subject variables. There was a statistically significant main effects of time $F(1, 74)= 22.602$, $p< .001$, $\eta_p^2= .234$ and emotion $F(5, 370)= 553.353$, $p< .001$, $\eta_p^2= .882$ and the time x emotion interaction was also significant $F(5, 370)= 9.958$, $p< .001$, $\eta_p^2= .119$, indicating that emotion ratings varied systematically over time. However, there were no significant interactions with tDCS or ABM (all $p>.2$) and therefore stimulation and/or ABM training did not affect VAS emotion ratings.

Acute state anxiety change: To assess acute state anxiety change STAI-S scores were analysed using rm-ANOVA with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (pre, post) as a within-subject variable. There was a significant main effect of time $F(1, 69)= 6.819$, $p= .011$, $\eta_p^2= .090$. However, there were no significant interactions with tDCS or ABM.

3.3.4. Questionnaire based measurements (follow up)

There was only moderate levels of compliance on follow up questionnaires and resulting loss of data. Therefore the sample size to be analysed at each follow up period dropped from 65 (baseline versus day 2) to 52 (baseline versus day 2, day 14 and day 28).

Trait anxiety follow up: Trait anxiety scores (STAI-T) over the follow up period were analysed using rm-ANOVA with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (baseline, day 2, day 14, day 28) as a

within-subject variable. There was no main effect of time on STAI-T scores and there were no significant interactions with tDCS or ABM (all p> .4). Therefore, the combination of tDCS and ABM did not significantly change trait anxiety over the follow up period.

BDI follow up: Depression scores (BDI) over the follow up period were analysed using rm-ANOVA with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (baseline, day 2, day 14, day 28) as a within-subject variable. There was no main effect of time on BDI scores and there were no significant interactions with tDCS or ABM (all p> .2). Therefore, the combination of tDCS and ABM did not significantly change depression scores over the follow up period.

PANAS follow up: PANAS scores in the follow up were analysed using rm-ANOVA with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (baseline, day 2, day 14, day 28) and valence (positive, negative) as within-subject variables. There was a significant main effect of time on PANAS scores $F(2.8, 133.7)= 5.891$, $p= .001$, $\eta_p^2= .109$, indicating that PANAS scores changed over the course of the follow up time. There was also a significant main effect of valence on PANAS scores ($F(1, 48)= 448.752$, $p< .001$, $\eta_p^2= .903$), reflecting higher positive PANAS scores and lower negative PANAS scores over all time periods. However, there were no significant interactions with tDCS or ABM (all p> .09). .

VAS follow up: An rm-ANOVA was carried out on the VAS emotion scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (baseline, day 2, day 14, day 28) and emotion (happy, sad, hostile, alert, anxious,

calm) as a within-subject variables. There was a significant main effect of emotion on VAS scores ($F(3, 127)= 280.522$, $p< .001$, $\eta_p^2= .862$), indicating higher VAS scores for happy, alert and calm (all means > 60) and lower VAS scores for sad, hostile and anxious (all means < 20). There were no other significant main effects or interactions with ABM or tDCS (all p>.1).

3.3.5. Behavioural measurements

Faces Dot probe Tasks: Face pairs were presented at two different durations, short (100ms) and medium (500ms), which were analysed independently. Analysis of short-duration trials aimed to test whether tDCS would abolish fear vigilance that is characteristic of rapid responses to threat seen in anxiety disorders. Analysis of medium-duration trials tested whether tDCS would abolish fear vigilance on a timescale similar to that of the ABM training.

Short duration dot probe task (100ms): As each arm of the current design involves some training manipulation there is no true sham condition with which to test for baseline effects. Therefore, the baseline healthy volunteer effect from Study One is assumed for the short trials.

To assess the effects of tDCS and training on emotional vigilance, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables, and emotion (happy, fearful) as a within-subject variable. As expected, there was a significant main effect of emotion $F(1, 74)= 6.204$, $p= .015$, $\eta_p^2= .077$, reflecting overall vigilance to fearful faces ($M= 13.60$, $SD= 58.87$) and avoidance of happy faces ($M= -6.62$, $SD= 45.31$). However, there were no

interactions with tDCS or ABM (all p> .3), indicating that emotional vigilance was not modified by the combination of tDCS and ABM.

Medium duration dot probe task (500ms): To assess the effects of tDCS and training on emotional vigilance at medium durations, rm-ANOVA was carried out on the medium trial vigilance scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables, and emotion (happy, fearful) as a within-subject variable. There was no main effect of emotion $F(1, 74)=1.144$, $p=.288$ reflecting overall negative vigilance scores for fearful ($M= -9.67$, $SD= 46.7$) and happy faces ($M=-18.95$, $SD=60.7$) at this duration. However, there were no significant interactions with tDCS or ABM (all p> .09). This suggests that also at medium duration trials, emotional vigilance was not modified by the combination of tDCS and ABM.

Dot probe words: 'Vigilance to threat' was operationally defined as the difference in reaction time to the probe when it was located behind the threatening word versus when the probe was located behind the neutral word, with positive scores representing greater vigilance to threat (faster reaction times) for the threatening word compared to the neutral word and negative scores representing less vigilance (slower reaction times) for the negative word compared to the neutral word.

To assess the effect of tDCS and ABM, ANOVA was carried out on the 'vigilance to threat' scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as fixed factors. The main effects of tDCS or ABM were not significant (all p >.2). The two-way tDCS x ABM interaction was also not significant $F(1, 74)= .967$, $p=.329$. Therefore, vigilance to threat was not affected by the combination of tDCS and ABM. Because the

stimuli in this task matched the training stimuli, it is the most likely candidate to show a training effect of ABM. To test for a training effect of ABM, the analysis was repeated using only the sham tDCS group. There was no effect of ABM on the 'vigilance to threat' scores $F(1, 37)= .622$, $p=.435$, meaning that vigilance to threatening words was not modified by the ABM training received.

Continuous Flash Suppression: 'Emotional vigilance' was operationally defined as the difference in reaction time to the face that emerged from the Mondrian pattern when it was emotional (happy/fearful) versus when the face was neutral, with positive scores representing greater vigilance (faster reaction times) for the emotional face compared to the neutral face and negative scores representing less vigilance (slower reaction times) for the emotional face compared to the neutral face.

To assess the effect of tDCS and ABM, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and emotion (happy, fearful) as a within-subject variable. As expected, there was a significant main effect of emotion $F(1, 71)= 29.880$, $p< .001$, $\eta_p^2= .296$, reflecting vigilance for fearful faces ($M= 84.9$, $SD= 235.35$) and avoidance of happy faces ($M= -133.4$, $SD= 203.99$). The main effects of tDCS and ABM were not significant (all $p> .1$). There were no other significant two-way or three-way interactions between emotion, tDCS or ABM (all $p> .6$), meaning that emotional vigilance was not was not modified by the combination of tDCS and ABM.

Instructed worry: The outcome measures in the mood/ state anxiety induction characterise changes resulting from the instructed worry phase. The measures are

differences in VAS scores before and after instructed worry and number of intrusions (both general intrusions and negative intrusions) before and after instructed worry.

To assess the effect of mood/state anxiety induction on VAS emotion scores, an rm-ANOVA was carried out on the VAS emotion scores with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and worry (pre-worry, post-worry) as within-subject variables. As expected, there was a significant main effect of worry $F(1, 73)= 11.007$, $p= .001$, $\eta_p^2= .131$, indicating that VAS emotion scores changed as a result of the mood/ state anxiety induction. As additionally expected, there was a statistically significant main effect of emotion ($F(2.7, 194.7)= 244.644$, $p< .001$, $\eta_p^2= .770$, indicating that specific VAS emotion scores varied systematically across emotions. There was no main effect of ABM $F(1, 73)= 0.027$, $p= .871$. However, there was a statistically significant two-way worry x ABM interaction $F(1, 73)= 5.919$, $p= .017$, $\eta_p^2= .075$, with the avoid threat ABM groups showing a mean increase in VAS scores across all emotions (positive and negative) compared to the attend threat group. There were no significant interactions with tDCS (all $p> .06$).

As VAS scores measure different emotions, some of which are positive and some of which are negative, additional analyses were carried out on the individual VAS scores, to clarify the nature of any potential effect of tDCS and ABM.

To assess mood/ state anxiety induction for different emotions an rm-ANOVA was carried out on the VAS scores for each emotion separately with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and worry (pre-worry,

post-worry) as a within-subject variable. As expected, there were statistically significant main effects of worry for VAS ratings of happy, sad, hostile, anxious and calm (all p< .002), indicating that VAS emotion scores changed as a result of the instructed worry (see Fig. 3.3.5.1.). There were no other main effects or interactions between worry, tDCS and ABM (all p> .09). There was a trend main effect of tDCS on VAS anxiety scores (F(1, 73)= 3.700, p= .058, η_p^2= .048), reflecting decreased VAS anxiety in the active tDCS group pre and post worry but no interactions with time or ABM.

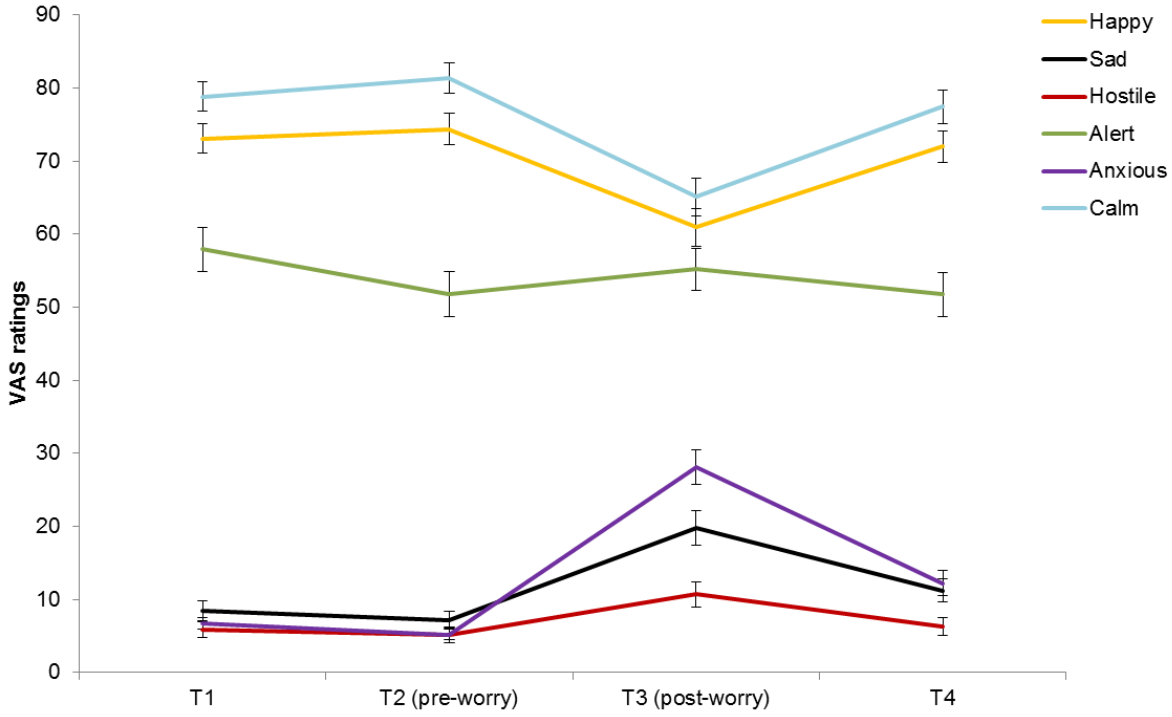

Figure 3.3.5.1. Changes in visual analogue scale (VAS) scores for different emotions over the course of the instructed worry task. T1: before task, T2: after first breathing focus, T3: after instructed worry period, T4: after second breathing focus. Scores are collapsed across tDCS/training groups. Error bars represent +- 1 standard error of the mean.

To assess the effect of state anxiety induction on thought intrusions, an rm-ANOVA was carried out on number of intrusions with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. As expected, there was a statistically significant main effect of worry F(1, 73)= 32.458, p< .001, η_p^2= .308, reflecting a higher number of

intrusions after mood induction (M= 4.77 , SD= 3.21) than before mood induction (M= 3.55, SD= 2.35). However, there were no other main effects or interactions with ABM or tDCS (all p> .3).

As negative thought intrusions were of particular interest the analysis was repeated for the negative thought intrusions only. An rm-ANOVA was carried out on number of negative intrusions with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically significant main effect of time $F(1, 73)= 16.257$, $p< .001$, $\eta_p^2= .182$, reflecting a higher number of negative intrusions after instructed worry (M= 1.25, SD= 1.83) than before instructed worry (M= 0.48 , SD= 0.93). However, there were no other main effects or interactions with ABM or tDCS (all p> .1).

Finally, the ratings (on a scale of 1-9, with 1 representing most negative and 9 representing most positive) of intrusions were analysed and the analysis was repeated for the ratings scores of the intrusions. An rm-ANOVA was carried out on the intrusion ratings with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically significant main effect of worry $F(1, 61)= 15.596$, $p< .001$, $\eta_p^2= .204$, reflecting more negatively rated intrusions after instructed worry (M= 5.59 , SD= 1.27) than before instructed worry (M= 6.21 , SD= 1.29). However, there were no other main effects or interactions with ABM or tDCS (all p>.1).

In sum, this analysis indicates that the VAS mood ratings and number/valence of intrusions is changed by mood induction via instructed worry but that this change is not modified by the combination of tDCS and ABM.

Fishing task: To assess performance in the fishing game an ANOVA was carried out on the number of fish caught with tDCS (active, sham) and ABM (attend threat, avoid threat) as the fixed factors. There were no main effects of tDCS or ABM on the number of fish caught (tDCS: $F(1, 75)= 1.537$, $p= .219$; ABM: $F(1, 75)= .190$, $p= .664$) and the two-way ABM x tDCS interaction was also not significant ($F(1, 75)= .443$, $p= .508$). This indicates that the number of fish caught was not modified by tDCS, ABM or a combination of the two.

3.4. Discussion

The measures used in this study failed to reveal any statistical interactions between tDCS and ABM training on attentional bias and mood. This is contrary to Clarke et al.'s (179) finding that anodal tDCS leads to greater evidence of attentional bias modification, using the same ABM training. Evidence from the sham tDCS group in the present study indicates that no basic training effect was observed. It is observed in the literature that the cognitive tasks used in ABM do not always successfully modify attentional biases (225, 226). In fact, Clarke et al. did not find a basic training effect in their sham tDCS condition, although this was achieved with the tDCS group. However, it is observed that it is only when the bias modification is successfully achieved that the subsequent mood effects reliably emerge (227). Therefore the improvement of bias modification procedures to increase the reliability of their effects is key to the advancement of the field. Although there has been some refinement of ABM parameters using results from a meta-analysis of

ABM effectiveness (47), this is an important question for future ABM research. However, there are also some design differences between the present study and Clarke et al., which merit further discussion.

The Clarke et al. study used a different electrode montage to the present study. The present study used a bipolar balanced DLPFC stimulation montage, as informed by the parameter optimisation in Study One. Clarke et al. used a unipolar montage with an extra-cephalic reference (cathodal) electrode on the neck. Both montages stimulate the left DLPFC with the anodal electrode. However, the present study also stimulated the right DLPFC with the cathodal electrode. It is also possible that the difference in electrical field and current density/directionality from these two montages could produce different effects on cortical excitability at locations distal from the electrode location. In addition, Clarke et al. used 1mA current strength in their study, whilst the present study used 2mA based on a review of the depression literature. The variability of effects of tDCS at different current strengths is still under evaluation, with a recent study indicating that 20 mins of 2mA cathodal motor cortex stimulation actually had excitatory effects on motor evoked potentials, whereas 20 mins of 1mA cathodal stimulation had inhibitory effects (201). However, the overarching present programme of research necessitates that 2mA of current is used in this study, in order to keep the investigation consistent with parameters in most recent clinical trials (156).

The Clarke et al. study also used differences from baseline to measure changes in attentional bias whereas the present study did not take baseline measurements and therefore comparisons were made between groups, reducing the statistical power of the present study.

The lack of a true control condition (with placebo bias modification training) is an important limitation of this study, as it removes the ability to make baseline measurements of attentional bias from which to compare the active conditions. This limitation could also be addressed by taking baseline measurements of attentional bias before stimulation and training. The present study avoided baseline measurements to reduce habituation to the task stimuli (particularly faces), which reduces their sensitivity (228). Additionally, in a single session of training, an *attend threat* control condition was predicted to produce a greater separation in bias modification from the *avoid threat* condition than no bias modification. However, a subsequent study (Chapter Five) incorporates baseline measurements and a true control condition (with no bias modification) in order to more directly compare results with the literature.

The vigilance reduction in the faces-dot probe task induced by DLPFC tDCS in Study One was not found in any of the active tDCS conditions in this second study. This requires some discussion. The extensive dot probe exposure necessary for completing the ABM training module may have reduced sensitivity to subsequent dot probe measurements. It is also possible that the ABM training intervention interfered with the reduction in vigilance previously observed (in Study One) when tDCS was given at rest. Aside from a recent pilot study indicating that concurrent cognitive control training (229) may augment its antidepressant efficacy, clinical investigations of tDCS for mood disorders have traditionally focused on delivering stimulation at rest (for a review see (157, 230)). Perhaps the 'at rest' factor is important to the clinical effects observed. Theoretically this could be explored by speculating on the potential interactions between tDCS and concurrent neural activity. For example, when a participant is undertaking a

task that engages the DLPFC, it is more likely that DLPFC neurons will be engaging in electrical signals (action potentials), meaning that these neurons will be depolarising. There is a chance that applying very weak direct current to already depolarising neurons will have little effect on their excitability, as membrane potential will already be fluctuating in this active state. On the other hand, applying weak electrical current to 'resting' DLPFC neurons could potentially induce resting membrane potential changes that may result in excitability modulation. Although resting state functional imaging does not currently implicate the DLPFC in a default mode (resting) networks (231), we have no way of knowing if the DLPFC is indeed inactive at rest. Therefore this is a speculative observation but could be examined practically by comparing behavioural effects of tDCS given at rest to tDCS given during a training paradigm. This comparison shall be made in a subsequent pooled analysis chapter (Chapter Six), utilising data from the first four experimental chapters of this thesis.

As discussed in Chapter One, a healthy volunteer experimental medicine model provides a useful way to refine parameters ahead of a large scale trial. However, it is possible that the effects of an intervention may be more readily observed in a clinical group, where the negative biases will be more pronounced. A subsequent study (Chapter Five) aims to address this by applying these interventions to an anxious participant group.

In terms of information yield from the present study, an instructed worry task was usefully modified to be delivered autonomously. This instructed worry task was a computerised version of a task that otherwise required two experimenters to execute (222). Although there were no effects of tDCS or ABM on performance in this task , it showed

good internal reliability as a lab based mood induction in healthy volunteers and could be usefully employed in future studies of worry and state anxiety.

As indicated in Study One, behavioural measurements can reveal important cognitive mechanisms which may underlie treatment effects. However, the use of multiple measures provides a more complete examination of the effects of an intervention. In particular, when examining the effects of an intervention on stress and state anxiety, the hormone cortisol provides a useful basis of assessment. This hormone, which is linked to stress and vulnerability to mood disorders, is easily measured and may provide a more objective and sensitive measure of stress and its modification. This is explored within this paradigm in the next experimental chapter.

Chapter Four

An exploration of the effects of frontal cortex stimulation and attentional bias modification on cortisol awakening response

4.1. Introduction

An additional arm of Study Two (Chapter Three) examined the effects of tDCS and ABM on hypothalamic-pituitary-adrenal (HPA) axis activity. HPA axis activity is governed by the secretion of adrenocorticotrophic hormone releasing factor (CRF) and vasopressin (AVP) from the hypothalamus, which in turn activates the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary, which finally stimulates the secretion of the glucocorticoids (cortisol in humans) from the adrenal cortex. Glucocorticoids then interact with their receptors in multiple target tissues including the HPA axis, where they are responsible for feedback inhibition both on CRF and AVP from the hypothalamus and directly on secretion of ACTH from pituitary corticotropes (232) (see Fig. 4.1.1.).

The HPA axis and therefore cortisol levels are very responsive to stress (233) and high cortisol levels are linked to various psychiatric disorders (234), as well as increased size and activity levels of the of the pituitary and adrenal glands in these populations (for a review, see (235)). The cortisol awakening response (CAR) is characterised as the increase in cortisol (measured in saliva) from the waking baseline into the first hour of wakefulness. The CAR is a reliable marker of HPA axis activity and is increased by work

stressors (236), related to an acute depressive state (237) and elevated in young people with familial risk of depression (238).

Antidepressant treatment reduces CAR in patients (239) and recent findings suggest that probiotic treatment reduces vigilance to threatening information and CAR (240) in healthy volunteers. Furthermore, repeated administration of an attentional bias modification (ABM) intervention reduces depressive symptoms and CAR reactivity in remitted depressed patients, suggesting a protective effect of this cognitive intervention (48). Therefore, CAR is a potentially useful additional measure of the effects of tDCS and ABM on cognition relevant to anxiety disorders and depression. Acute effects of tDCS on cortisol have been observed (241, 242) but the CAR has not been examined in tDCS research to date. Thus, the current investigation examined the effects of tDCS and ABM on HPA axis activity by measuring changes in the cortisol awakening response and mean levels of cortisol.

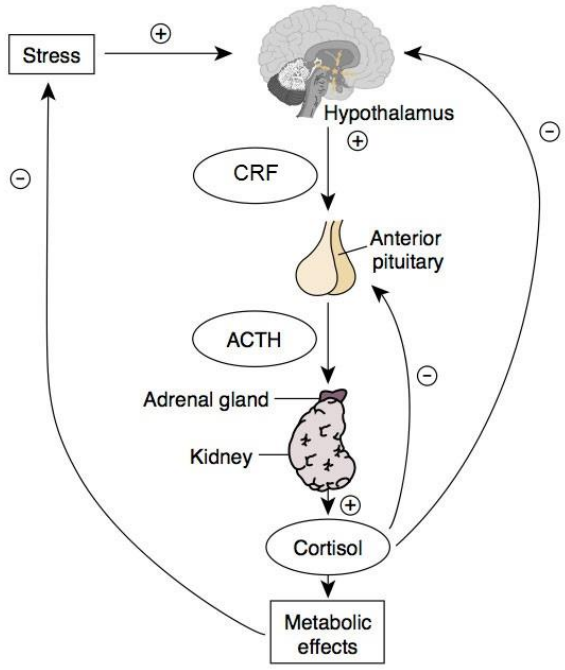

Figure 4.1.1. The HPA Axis. Reproduced with permission (243)

4.2. Methods and materials

4.2.1. Subjects

This investigation was carried out in the same group as Study Two (Chapter Three). Ethical approval and screening procedures were as per Study One (Chapter Two).

4.2.2. Design

A four arm, between-groups design was carried out as per Study Two (Chapter Three).

4.2.3. Salivary cortisol

HPA axis activity was assessed on the morning of testing (before tDCS/ABM) (day 1), the morning after testing (day 2) and at four week follow up (day 28), using the salivary cortisol awakening response (236). Each participant was given three saliva collection kits, which included written instructions (see Appendix III), three saliva tubes (salivettes, Sarstedt Ltd., Nümbrecht, Germany), and packaging and postage materials. At the screening session participants received a demonstration of the salivettes, and were shown how to place the cotton tube in their mouth and move it around to collect the saliva. For each CAR measurement, participants were instructed to provide three saliva samples with the salivettes, taken themselves in their own home immediately upon waking and subsequently every 15 min until 45 min post-waking. Saliva samples were delivered in person or sent by first class post and once received, stored at 4 °C prior to analysis. Cortisol was measured using a commercial ELISA (Salimetrics Europe Ltd., Newmarket, UK) colorimetric competitive enzyme immunoassay kit, within 7 days of sample collection after which samples were destroyed.

4.2.4. TDCS

As described in Study Two (Chapter Three).

4.2.5. ABM module

As described in Study Two (Chapter Three).

4.2.6. Calculations and Statistics

Cortisol awakening response was calculated by subtracting the first sample (baseline waking sample) from the higher of the two subsequent samples. Relative measurements of CAR were also analysed by dividing the absolute CAR by the first sample to give a percentage change, thereby controlling for baseline differences. In addition, post-hoc analyses examined mean cortisol levels on each day. All data were analysed using rm-ANOVAs, with time (day 1, day 2, day 28) as a within subjects variable and tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables. Significant interactions were followed up with planned contrasts to compare the effects of tDCS and ABM separately. Where assumptions of equality of variances were broken, a Huynh-Feldt correction was applied to degrees of freedom. The experimenter was not unblinded to the tDCS/ABM conditions until the main analysis had been completed.

4.3. Results

Compliance was moderate in this study, with the number of subjects' data decreasing from 70 (day 1) to 61 (day 2) to 52 (day 28). Only 45 subjects returned all three saliva sample sets.

4.3.1. Group matching

As per Chapter Three. Group matching on age, gender, education and baseline measures of depression (BDI) and trait anxiety (STAI-T) was maintained after attrition from drop out at day 2 and day 28 follow up (all $p>.1$).

4.3.2. CAR – Absolute measurements

To assess the effect of tDCS/ABM on CAR changes the day after testing and at four week follow up an rm-ANOVA was carried out on the absolute CAR measurements with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (day 1, day 2, day 28) as within-subject variable. There were no significant main effects of time or tDCS (all $p> .1$) on absolute CAR but there was a main effect of ABM $F(2, 82)= 5.564$, $p= .023$, $\eta_p^2= .119$. However, there were no interactions of time with tDCS or ABM (all $p>.1$). The significant effect of ABM was followed up with separate one-way ANOVAs of the absolute CAR measurements at each time period (baseline, day 2, day 28) with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables. There were no baseline differences between the tDCS/ ABM groups on absolute CAR measurements (all $p>.1$). However, there was a trend effect of tDCS on CAR measurements at day 2, with the sham tDCS group showing higher absolute CAR ($M= 10.68$, $SD= 10.57$) than the active tDCS group ($M= 4.76$, $SD= 10.13$), $F(1, 41)=$

3.821, p= .057, η_p^2= .085 (Fig. 4.3.2.1.). There were no effects of ABM on absolute CAR at day 2 follow up and there were no effects of tDCS or ABM on absolute CAR at 28 day follow up (all p>.1).

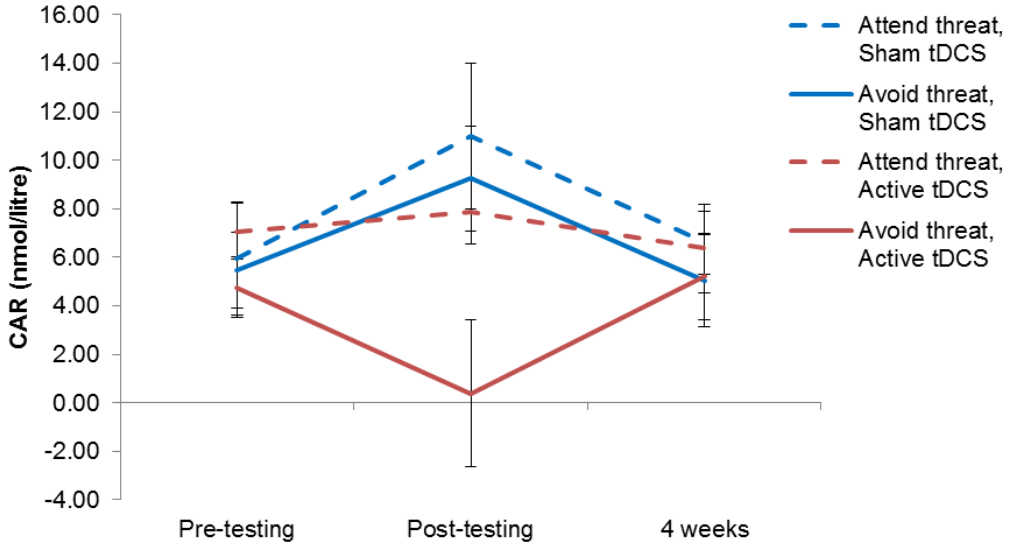

Figure 4.3.2.1 Changes in absolute (nmol/litre) cortisol awakening response (CAR) over the course of the 4 week follow up period (testing day, day after testing and 4 weeks after testing). CAR is characterised as the difference between cortisol levels at awakening and their peak within the first hour of awakening. Error bars represent +- 1 standard error of the mean.

4.3.3. CAR- Relative measure

To assess the effect of tDCS/ABM on relative CAR changes the day after testing and at four week follow up an rm-ANOVA was carried out on the participants relative CAR measurements (individual CAR/individual baseline cortisol, expressed as a %) with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (day 1, day 2, day 28) as a within-subject variable. The main effect of time was not significant F(2, 82)= .244, p= .784, but there was a main effect of tDCS F(1, 41)= 4.874, p= .033, η_p^2= .106. However, there were no interactions of time with tDCS or ABM (all p> .2). The significant effect of tDCS was followed up with separate one-way ANOVAs of the relative CAR measurements at each time period, with tDCS (active, sham) and

ABM (attend threat, avoid threat) as between-subjects variables. There were no baseline differences between the tDCS/ ABM groups on relative CAR measurements (all p> .8). However, there was an effect of tDCS on relative CAR measurements at day 2, with the sham tDCS group showing higher relative CAR (M=164%, SD=159) than the active tDCS group (M=64%, SD=83) F(1, 41)= 7.403, p= .010, η_p^2= .153 (Fig. 4.3.3.1.). There were no effects of ABM at day 2 follow up and there were no effects of either tDCS or ABM on relative CAR at 28 day follow up (all p> .1).

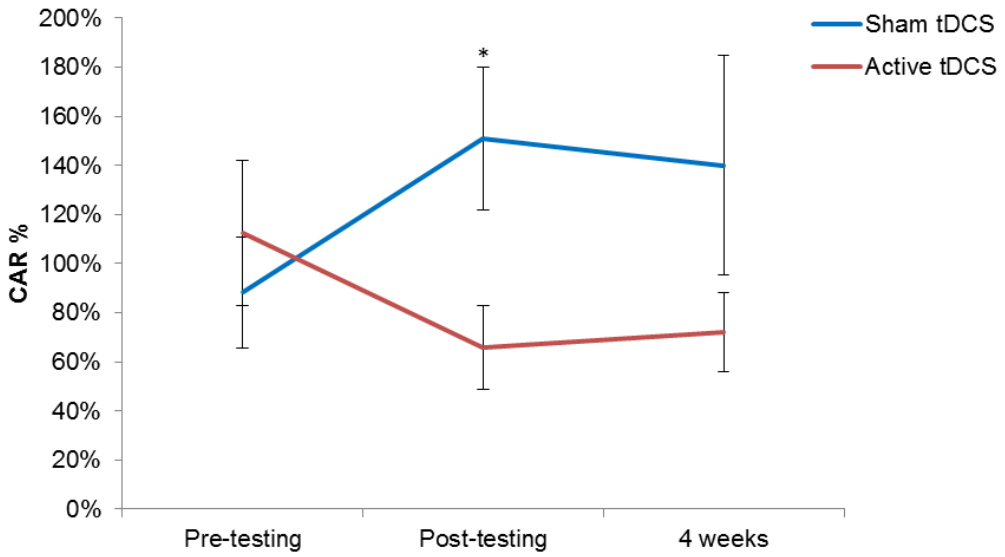

Figure 4.3.3.1. Changes in relative cortisol awakening response (CAR) over the course of the 4 week follow up period (testing day, day after testing and 4 weeks after testing). Scores are collapsed across ABM conditions. Error bars represent +- 1 standard error of the mean.

4.3.4. Mean cortisol levels

In addition to examining the cortisol awakening response, mean cortisol levels were analysed in a post-hoc exploration. To assess the effect of tDCS/ABM on mean cortisol levels the day after testing an rm-ANOVA was carried out on mean cortisol levels with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables and time (day 1, day 2, day 28) as a within-subject variable. The main effect of time was not significant F(2, 82)= .132, p= .876 and there were no interactions with tDCS or ABM (all p>.09).

4.4. Discussion

A single session of tDCS (regardless of ABM condition) was found to reduce subsequent cortisol awakening response (CAR) on the morning after stimulation. This was a statistically significant effect when measuring relative CAR and a trend when measuring absolute CAR. There were no main effects of ABM or interactions between tDCS and ABM on any of these follow up measures of cortisol.

The strongest findings appear to suggest that tDCS reduced relative cortisol awakening response the morning after testing. The direction of change is similar to acute reductions in cortisol seen after a single session of tDCS in healthy volunteers (242) and in volunteers with maths anxiety (241). It is possible that this decreased CAR could be a biological correlate of the reduction in threat vigilance seen in earlier studies of tDCS (Chapter Two) and/or antidepressant effects of tDCS in clinical trials (156). This reduction in CAR is similar to effects seen with antidepressant drug treatment (239). In addition, reductions in vigilance to threat and CAR have been associated with probiotic treatment (240) in healthy volunteers and a two week ABM intervention in remitted depressed patients (48). Taken together these results suggest that CAR may be a potentially useful biomarker to consider for the prediction and measurement of tDCS efficacy in clinical groups.

The effects of ABM on cortisol appeared quite weak in this study, which is unsurprising given the ABM intervention was unsuccessful in modifying attentional bias or mood/ state anxiety (Chapter Three). Previous effects of ABM on CAR have been

found with face and not word based ABM (48) and additionally, after repeated sessions of ABM (daily over two weeks), rather than a single session.

Caution must be exercised when interpreting the results for tDCS, especially as the dearth of behavioural effects in the same investigation (see Chapter Three) means it is not possible to relate the CAR changes to changes in behaviour. However, perhaps the behavioural measurements used in Study Two were not sensitive enough to detect an effect, particularly after exposure to the emotional ABM stimuli. Possibly cortisol measurements can be employed more usefully acutely in tDCS studies, as other studies have shown that anodal DLPFC tDCS may decrease cortisol levels acutely (241, 242). In addition, the use of a healthy sample may have reduced the sensitivity of this measure and it could be more usefully employed in patient or 'at risk' groups which are characterised by higher CAR at baseline (234). Finally, the small sample size and moderate levels of compliance may have reduced the power of this measure to detect a potentially stronger effect. In summary, findings from this initial study suggest that tDCS may have effects on HPA axis reactivity. To test whether this is related to clinical effects of this intervention, it would be interesting to examine acute changes in CAR in subsequent responder versus non-responder groups in clinical trials and future studies (particularly clinical trials) may benefit from the inclusion of this measure.

Chapter Five

A further investigation into the effects of frontal cortex stimulation and attentional bias modification at the University of Western Australia

5.1. Introduction

The prior study (Study Two) reported in Chapter Three was completed as part of a collaboration between the authors and a group from the University of Western Australia (UWA). The results reported in Chapter Three differed to the prior findings of the UWA group (179) using a similar design. However, there were some differences in parameters used that could explain the non-replication. Therefore to investigate this further the author carried out an additional investigation at UWA.

The first parameter which differed between the two studies was electrode montage used. The UWA group used an unipolar montage which placed the anode on the left dorsolateral pre-frontal cortex (DLPFC) and the cathode on the left superior region of the trapezius muscle near the base of the participant's neck, whereas Study Two employed a bipolar balanced montage, with the anode on the left DLPFC and the cathode on the right DLPFC, following the parameter optimisation in Study One (Chapter Two). Secondly, the current strength (2mA compared to 1mA) and electrode size ($25cm^2$ compared to $28cm^2$) differed between the two studies, resulting in different current densities. Finally, the testing parameters differed as the UWA study included baseline measurements of vigilance

and hence calculated change scores after ABM/training, whereas the Study Two did not include baseline measurements.

For the present investigation the authors kept some of the parameters of the prior tDCS/ABM study (Study Two). This included the bipolar balanced electrode montage, as this had been the result of parameter optimisation in Study One, had produced reduced vigilance to threat in Study One and best matched the electrode montage used in clinical trials of tDCS for depression. In addition, 2mA of current was kept consistent with Study Two to best match common parameters in clinical trials. The instructed worry task was also retained from Study Two as it had shown reliable mood induction and held promise for use in more sensitive samples. The dot probe measurements included were a prior feature of both Study Two and the UWA study. However, to avail of the experience of the UWA group with ABM techniques, a neutral training condition was included in this investigation in order to provide a true 'sham' condition and baseline measurements included in the design to enable easier comparison to the prior findings from UWA. Furthermore, it was decided, instead of healthy controls, to recruit a high anxious sample as anxious subjects have greater attentional bias to threat (102, 103) and therefore may provide a larger range of threat vigilance to modify, as well as providing more clinical relevance.

5.2. Methods and materials

5.2.1. Subjects

Ethical approval was obtained from the UWA Human Research Ethics Office (RA/4/1/4088). Participant selection was guided by pre-screening of 839 individuals from the UWA School of Psychology research participant pool on the trait version of the State-

Trait Anxiety Inventory -Trait (STAI-T). Persons who had a STAI-T score of more than 45 were invited to sign up for the study. Trait anxiety scores of 40 or over are proposed to represent clinical levels of anxiety in non-elderly adult populations (244) and a score of 45 is estimated to be at the 67th population percentile for this group (245). 72 participants signed up for the study and were screened in person with a reduced version of the structured clinical interview for DSM-IV disorders.. One participant was excluded because of current antidepressant medication, another because of current depressive episode and another participant terminated the stimulation early because of discomfort. This left 69 remaining participants (aged 17-31, 43 female). Participants were compensated for their time with course credits or at rate of AUD$10 per hour.

5.2.2. Design

A four arm, between-groups design was carried out. This involved 17 participants receiving active tDCS and 'avoid threat' ABM, 17 participants receiving active tDCS and 'neutral' ABM, 19 participants receiving sham tDCS and 'avoid threat' ABM and a further 16 receiving sham tDCS and 'neutral' ABM (see Table 5.2.2.1).

Group	**tDCS** (Bipolar-balanced DLPFC)	**ABM**
Group 1	2mA, 20 mins	avoid threat
Group 2	2mA, 20 mins	neutral
Group 3	sham, 20 mins	avoid threat
Group 4	sham, 20 mins	neutral

Table 5.2.2.1. Factorial, four arm design of stimulation and training group assignment, counterbalanced and gender matched across groups.

On the day of the study participants filled out some questionnaires before being prepared for stimulation. The electrodes (25cm²) were placed in saline soaked sponges and affixed to the scalp with a rubber band. Before the stimulation device was turned on, participants carried out baseline computerised emotional processing tasks (described below). After the baseline tasks had been completed, the stimulation (bipolar-balanced DLPFC, 20 minutes at 2mA) was applied whilst participants completed a computerised ABM training module (stimulation and training type defined by group allocation). The experimenter was blind to the ABM condition. After the tDCS/ABM participants were asked to carry out a series of computerised emotional processing tasks (described below) and fill in the mood scales again. In addition, a lab based stressor (instructed worry task) was used to measure the effects of tDCS/ABM on induced mood/ state anxiety.

5.2.3. Transcranial Direct Current Stimulation (tDCS)

Stimulation was delivered using a battery-powered, current-controlled iontophoresis device (Dual Channel Iontophoresis System, Chattanooga, Hixon, TN (246)). As described in Chapter Two/ Chapter Three the rubber electrodes (25cm²) were placed in saline soaked sponges and affixed to the scalp with a rubber band. The bipolar-balanced electrode montage was employed which had the anode (positive) electrode on the left DLPFC and the cathode (negative) electrode on the right DLPFC (F3 and F4 respectively, in the 10/20 system of electrode placement). Stimulation (20 minutes at 2mA) was applied while the participant completed the ABM training module. In the sham condition participants received 30 sec of direct current after which the device was turned off without the participants' knowledge. This method of sham stimulation produced the physical sensations typical of active tDCS but did not allow double blinding of the experimenter.

5.2.4. ABM module

The attentional bias training procedure was as described in Chapter Three, with one alteration. Instead of an attend threat control condition participants had a neutral training condition where they were presented with two neutral words and the probe in a random location. This was intended to provide a true sham training condition with no bias modification.

5.2.5. Questionnaire measures

Participants filled out baseline mood questionnaires, including the State-Trait Anxiety Inventory (STAI) (24) and a Visual Analogue Scale (VAS) of emotions (Happy, Sad, Hostile, Alert, Anxious, Calm). The VAS measures were repeated after stimulation/training. The VAS measures were again repeated four times during the instructed worry task. Participants were asked to fill in online versions of the STAI the day after testing, at two week follow up and at four week follow up.

5.2.6. Behavioural measurements

The behavioural tasks were carried out in a predetermined order (see Fig 5.2.6.1), so that the potential effects of tDCS decay could be held as constant as possible. The ABM training and all behavioural tasks were programmed in e-prime stimulus presentation software (E-prime 1.2, Psychology Software Tools, USA(185)).

Vigilance to threat was assessed using the dot probe faces task, as used in Study Two (described in Chapter Three). Vigilance to threat was also assessed using a word based version of the dot probe task. This was almost identical in format to the ABM

training module and as described in Chapter Three. Worry persistence was assessed using a computerised instructed worry task, as described in Chapter Three.

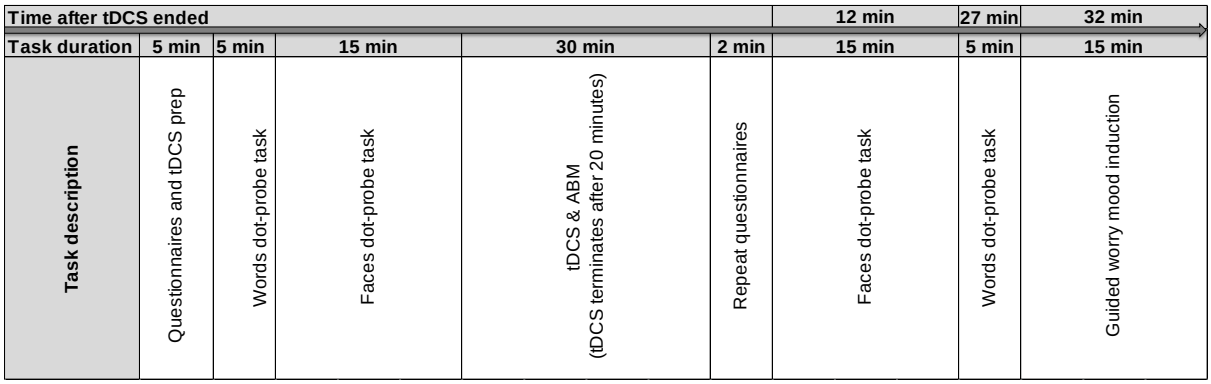

Figure 5.2.6.1. Timeline of tDCS/ABM and behavioural tasks. Values above the arrow denote time elapsed after tDCS (min), values below the arrow denote task duration (min).

5.2.7. Calculations and Statistics

Accuracy scores and individual means of reaction times were calculated for each valence (threat, neutral) in the dot probe words task. The faces dot probe task required calculation of an index of emotional vigilance, as per Study One and Study Two. Only correct trials were included in the reaction time calculations. Extreme outlying data was removed from all psychological tasks as per Study One and Study Two. This resulted in less than 3.5% of data being lost as outliers on any task. For the instructed worry task, as per Study Two VAS change scores were calculated from the pre and post instructed worry VAS scores. There was also a count of negative intrusions in the breathing focus, pre and post instructed worry and a calculation of the mean valence of intrusions pre and post worry. All data were analysed using IBM SPSS Statistics 20 (192). Questionnaire data were analysed using two-way analyses of variance (ANOVAs) for baseline questionnaires and repeated measures (rm) -ANOVAs for repeated questionnaires, with questionnaire scores as the within-subject variable and tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables. Behavioural data from each of the tasks (including reaction

times and accuracy) were analysed using rm-ANOVAs, with time (pre and post tDCS/ABM), emotional valence of stimuli (positive, negative) and, (if relevant) stimuli duration (medium, short) as within subjects variables and tDCS (active, sham) and ABM (avoid threat, neutral) as between-subjects variables. Significant interactions were followed up with planned contrasts to compare the effects of tDCS and training separately. Where assumptions of equality of variances were broken, a Huynh-Feldt correction was applied to degrees of freedom. Multiple comparisons were corrected for within-tasks but not between-tasks. The experimenter was not unblinded to the ABM condition until the main analysis had been completed.

5.3. Results

5.3.1. Group matching

The groups were matched in terms of gender, age and highest education level (all $p>.1$). Baseline sub-clinical levels of trait anxiety (using the STAI-T) did not differ between the groups $F(3, 61)= .010$, $p= .999$.

5.3.2. Questionnaire based measurements (acute)

Acute VAS change: To assess acute emotion rating changes an rm-ANOVA was carried out on the VAS emotion change scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and time (pre, post) as within-subject variables. There was a statistically significant main effects of time $F(1, 57)= 18.633$, $p< .001$, $\eta_p^2= .246$ and emotion $F(2.8, 160.2)= 76.050$, $p< .001$, $\eta_p^2= .572$ and the time x emotion interaction was also significant $F(2.7, 155.5)= 9.958$, $p= .002$, $\eta_p^2= .090$, indicating that emotion ratings varied systematically

over time. However, there were no significant interactions with tDCS or ABM (all p> .1) and therefore stimulation and/or ABM did not acutely affect VAS emotion ratings.

5.3.3. Questionnaire measurements (follow up)

There were only moderate levels of compliance on follow up questionnaires and resulting loss of data. Therefore the sample size to be analysed at each follow up period dropped from 65 (baseline) to 57, 45 and 51 (day 2, day 14 and day 28).

State anxiety follow up: State anxiety scores (STAI-S) were taken at baseline, the day after testing and at 2 weeks follow up. These state anxiety scores were analysed using rm-ANOVA with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and time (baseline, day 2, day 14) as a within-subject variable. There was a significant main effect of time on state anxiety scores $F(1.6, 60.4)= 5.701$, $p= .009$, $\eta_p^2= .134$, and a significant main effect of tDCS ($F(1,37)= 7.685$, $p=.009$, $\eta_p^2=.172$. However, there were no interactions of time with tDCS or ABM (all p>.4). The main effect of tDCS was followed up with separate one-way ANOVAs of the STAI-S scores at each time period, with tDCS (active, sham) and ABM (attend threat, avoid threat) as between-subjects variables. There were no baseline differences between the tDCS/ ABM groups on STAI-S scores at baseline or at day 14 (all p> .1). However, there was a significant effect of tDCS on STAI-S scores at day 2, with the active tDCS group showing higher STAI-S scores (M= 43.74, SD= 9.88) than the sham tDCS group (M= 33.9, SD= 8.05), $F(1,37)= 12.014$, $p= .001$, $\eta_p^2= .245$ (see Fig. 5.3.3.1.). This suggests that tDCS increased state anxiety the day after stimulation. There were no effects of ABM on STAI-S scores at day 2 or day 14 follow up.

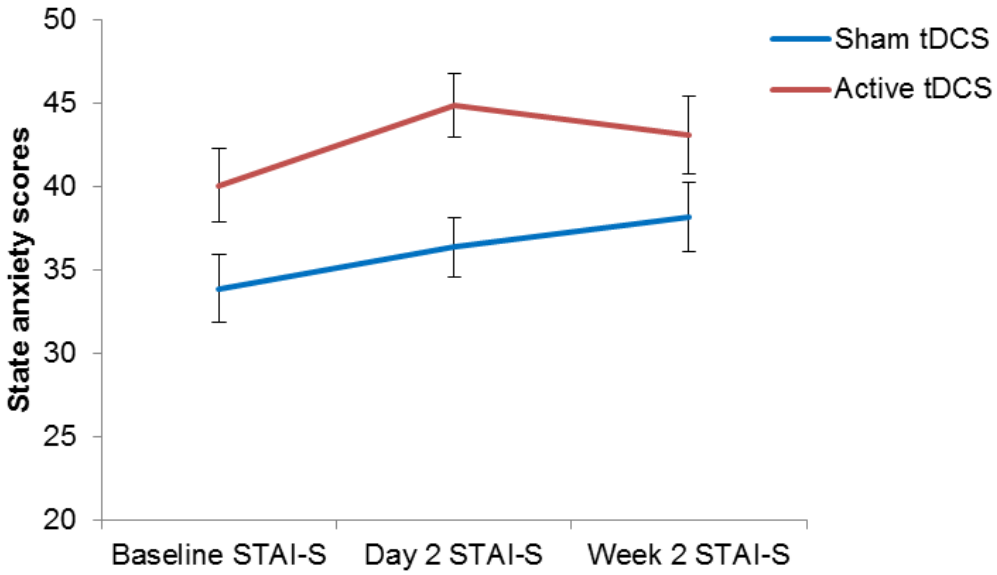

Figure 5.3.3.1. State anxiety scores follow up. Values denote mean STAI-S (baseline, day 2 and day 14) scores. Scores are collapsed across ABM conditions. Error bars represent +- 1 standard error of the mean.

Trait anxiety follow up: Trait anxiety scores (STAI-T) were taken at baseline and at 4 week follow up. These trait anxiety scores were analysed using rm-ANOVA with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and time (baseline, day 28) as a within-subject variable. There was a significant main effect of time on trait anxiety scores ($F(1.6, 60.4)= 5.701$, $p= .009$, $\eta_p^2= .134$), with lower trait anxiety scores at day 28 than at baseline. However, there were no main effects or interactions of time with tDCS or ABM. This suggests that the combination of tDCS and ABM did not modify trait anxiety at four week follow up.

5.3.4. Behavioural measurements

Faces Dot probe Tasks: 'Vigilance to threat' was operationally defined as in Chapter Two. Face pairs were presented at two different durations, short (100ms) and medium (500ms), which were analysed independently. Analysis of short-duration trials aimed to test whether tDCS/ABM would abolish fear vigilance that is characteristic of rapid

responses to threat seen in anxiety disorders. Analysis of medium-duration trials tested whether tDCS/ABM would abolish fear vigilance on a timescale similar to that of the training task.

Short duration dot probe task (100ms): Before testing for a change in behaviour with tDCS/training, it was first examined whether the expected fear vigilance phenomenon was present at baseline. Paired samples t-tests were carried out on the baseline reaction times, for fearful/neutral face pairs in the short duration task. Participants did not exhibit the expected pattern of fear vigilance, with similar reaction times when the probe was located behind the fearful face or the neutral face of a pair $t(68)= -1.021$, $p= .311$. This indicates that at baseline there was no significant difference in vigilance to fearful or neutral faces at short durations. Paired 1-sample t-tests (2-tailed, against zero) on the vigilance scores confirmed that, at baseline participants neither showed significantly greater vigilance towards fearful faces $t(68)= 1.021$, $p= .311$, nor significantly less vigilance towards happy faces $t(68)= -.399$, $p=.691$ at short durations.

To assess the effects of tDCS and ABM on emotional vigilance at short durations, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables, and time (pre-, post tDCS/ABM) and emotion (happy, fearful) as a within-subject variables. As per the baseline analysis, there was no significant main effect of emotion $F(1, 65)= 0.002$, $p= .962$ or time $F(1, 65)= 1.710$, $p= .196$, reflecting similar vigilance for fearful faces and happy faces pre and post tDCS/ABM. There were no interactions of time or emotion with tDCS or ABM (all $p> .150$), indicating that emotional vigilance at short durations was not modified by the combination of tDCS and training.

Medium duration dot probe task (500ms): Before testing for a change in behaviour with tDCS, it was first examined whether the expected fear vigilance phenomenon was present at baseline. Paired samples t-tests were carried out on the baseline reaction times, for fearful/neutral face pairs in the medium duration task. Participants did not exhibit the expected pattern of fear vigilance, with similar reaction times when the probe was located behind the fearful face or the neutral face of a pair t(68)= -1.214, p= .229. This indicates that at baseline there was no significant difference in vigilance to fearful or neutral faces at medium durations. Paired 1-sample t-tests (2-tailed, against zero) on the vigilance scores confirmed that, at baseline, participants did not show significantly greater vigilance towards fearful faces t(68)= 1.214, p= .229, or away from happy faces t(68)= -1.797, p= .077 at medium durations.

To assess the effects of tDCS and ABM on emotional vigilance at medium durations, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables, and time (pre-, post tDCS/ABM) and emotion (happy, fearful) as a within-subject variables. As per the baseline analysis, there was no significant main effect of emotion F(1, 65)= 2.399, p= .126 or time F(1, 65)= 2.046, p= .157), reflecting similar vigilance for fearful faces and happy faces at medium durations. There was a trend two-way emotion x time interaction F(1, 65)= 3.950, p= .051, η_p^2= .057, reflecting higher fear vigilance compared to happy vigilance at baseline (fear= 6.1; happy= -10.5), compared to retest (fear= -10.77; happy= -9.1) (see Fig. 5.3.4.1). This indicates that fear vigilance reduced at overall at retest, regardless of tDCS or ABM. There were no interactions with tDCS or ABM (all p>.1).

This suggests that at medium duration trials, fear vigilance may be reduced over time but emotional vigilance is not affected by the combination of tDCS and ABM.

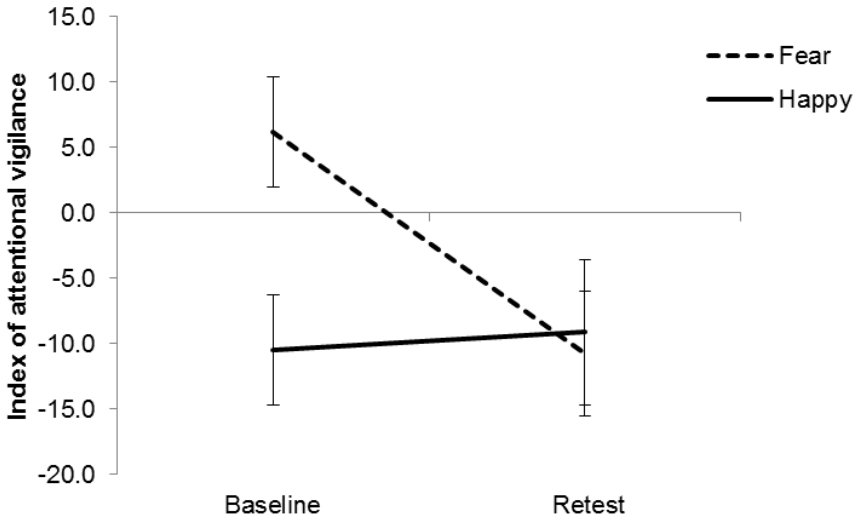

Figure 5.3.4.1. Effect of time on attentional vigilance to emotional faces at 500msec duration. Mean reaction times (ms) when the probe was located behind the fearful face of a fearful/neutral pair versus when the probe was located behind the neutral face of a fearful/neutral pair. Scores are collapsed across tDCS and ABM conditions. Error bars represent +- 1 standard error of the mean.

Dot probe words: 'Vigilance to threat' was operationally defined as in Chapter Three. Before testing for a change in behaviour with tDCS, it was first examined whether the expected fear vigilance phenomenon was present at baseline. Paired samples t-tests were carried out on the baseline reaction times, for threatening/neutral word pairs. Participants did not exhibit the expected pattern of fear vigilance, with similar reaction times when the probe was located behind the threatening word or the neutral word of a pair $t(68)= 1.114$, $p= .269$. Paired 1-sample t-tests (2-tailed, against zero) on the vigilance scores confirmed that, at baseline participants did not show significantly greater vigilance towards threatening words $t(68)= -1.114$, $p= .269$. This indicates that at baseline there was no significant difference in vigilance to threatening or neutral words.

To assess the effect of tDCS and ABM, rm-ANOVA was carried out on the 'vigilance to threat' scores with tDCS (active, sham) and ABM (neutral, avoid threat) as

between subjects factors and time (pre-, post tDCS/ABM) as a within subjects factor. The main effect of time was not significant F(1, 65)= 1.563, p= .216. There were no significant main effects or two-way interactions with tDCS or ABM (all p> .191).

To test for a training effect of ABM, the analysis was repeated using only the sham tDCS group. There were no main effects of time or interactions with ABM (all p>.7) meaning that vigilance to threatening words was not affected by the type of ABM received.

Baseline vigilant subgroups

Attentional bias modification can be thought to only have potential usefulness when there are baseline attentional biases present to be modified. With this in mind the analysis was repeated only for subjects who displayed net vigilance to threat at baseline in each task.

Short duration dot probe task (100ms): 37/69 (54%) of the participants tested had a positive vigilance to fearful faces at short durations at baseline. To assess the effects of tDCS and training on this baseline emotional vigilance, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables, and time (pre-, post tDCS/ABM) and emotion (happy, fearful) as a within-subject variables. For this group, there was a trend main effect of time F(1, 33)= 3.961, p= .055, η_p^2=.107, and the two-way time x emotion interaction was significant F(1, 33)= 7.058, p= .012, η_p^2= .176, reflecting lower fear vigilance post tDCS/ABM (see Fig. 5.3.4.2.). However, there were no interactions with tDCS or ABM (all p>.5), indicating that emotional vigilance at short durations was not modified by the combination of tDCS and ABM, even in this subgroup.

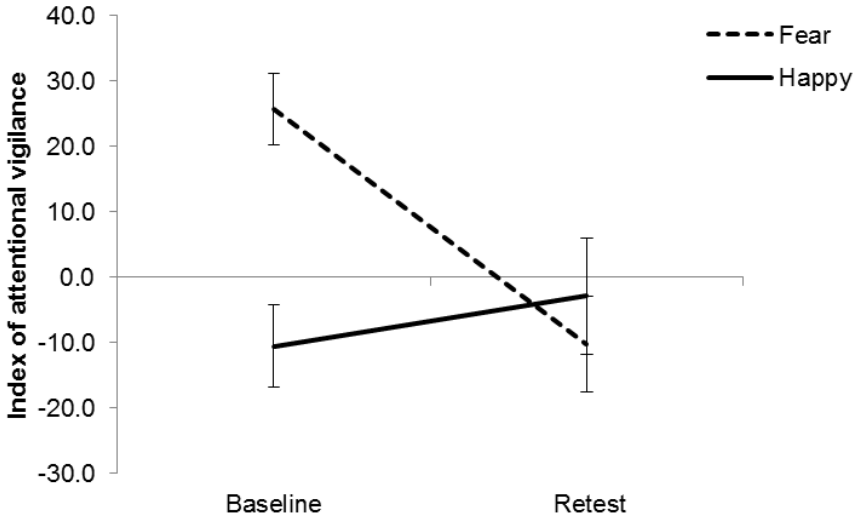

Figure 5.3.4.2. Effect of time on attentional vigilance to emotional faces at 100msec duration. Mean reaction times (ms) when the probe was located behind the fearful face of a fearful/neutral pair versus when the probe was located behind the neutral face of a fearful/neutral pair. Scores are collapsed across tDCS and ABM conditions. Error bars represent +- 1 standard error of the mean.

Medium duration dot probe task (500ms): 40/69 (58%) of the participants tested had a positive vigilance to fearful faces at medium durations at baseline. To assess the effects of tDCS and training on this baseline emotional vigilance, rm-ANOVA was carried out on the vigilance scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables, and time (pre-, post tDCS/ABM) and emotion (happy, fearful) as a within-subject variables. For this group, there was a significant main effect of emotion $F(1, 36)= 10.305$, $p= .003$, $\eta_p^2= .223$, reflecting higher fear vigilance at baseline, as the selection criteria required. However, there was also a significant main effect of time $F(1, 36)= 14.740$, $p< .001$, $\eta_p^2= .290$, and the two way time x emotion interaction was also significant $F(1, 36)= 7.627$, $p= .009$, $\eta_p^2= .175$, reflecting lower fear vigilance post tDCS/ABM (see Fig 5.3.4.3.).

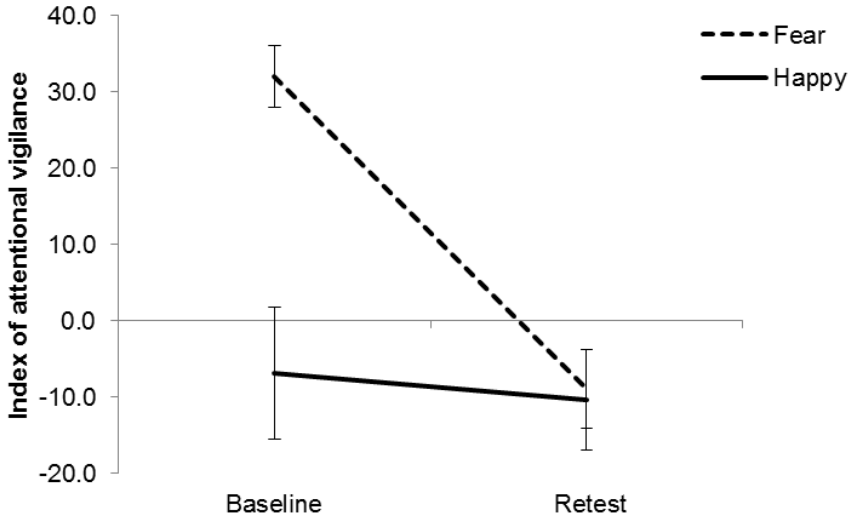

Figure 5.3.4.3. Effect of time on attentional vigilance to emotional faces at 500msec duration. Mean reaction times (ms) when the probe was located behind the fearful face of a fearful/neutral pair versus when the probe was located behind the neutral face of a fearful/neutral pair. Scores are collapsed across tDCS and ABM conditions. Error bars represent +- 1 standard error of the mean.

Dot probe words: 31/69 (44%) of the participants tested had a positive vigilance to threatening words at medium durations at baseline. To assess the effect of tDCS and ABM, rm-ANOVA was carried out on the 'vigilance to threat' scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between subjects factors and time (pre-, post tDCS/ABM) as a within subjects factor. There was a significant main effect of time $F(1, 27)= 14.760$, $p= .001$, $\eta_p^2= .353$ reflecting reductions in threat vigilance from pre to post tDCS/ABM. There were no significant two-way interactions with tDCS or ABM (all $p> .191$). There was a significant three-way time x tDCS x ABM interaction $F(1, 27)= 5.055$, $p= .033$, $\eta_p^2= .158$. This reflects larger reductions in threat vigilance (post – pre) for the active tDCS condition receiving the neutral ABM and the sham tDCS condition receiving the avoid threat ABM (see Fig. 5.3.4.4.).

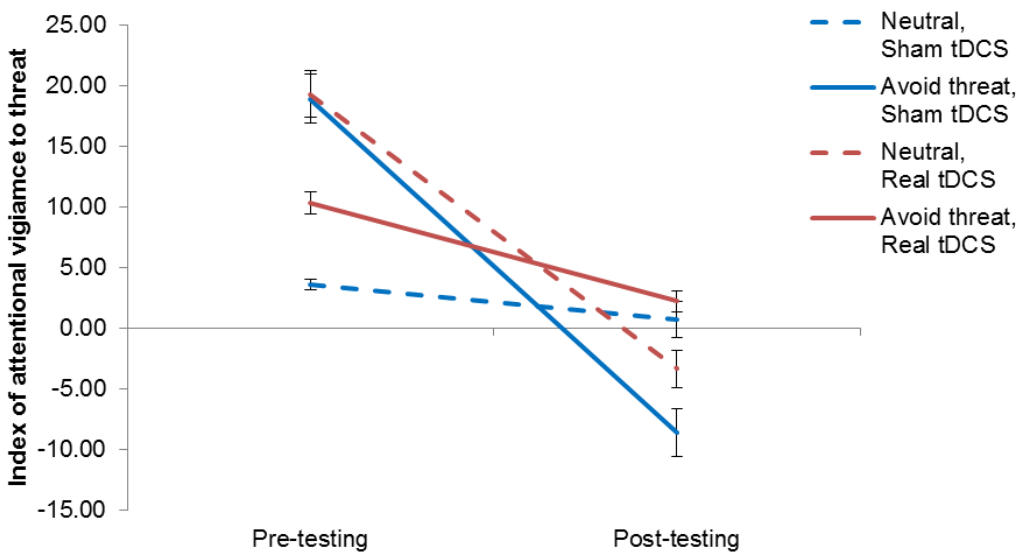

Figure 5.3.4.4. Effect of tDCS and ABM on attentional vigilance to threatening words. Mean reaction times (ms) when the probe was located behind the threatening word versus when the probe was located behind the neutral word. Error bars represent +- 1 standard error of the mean.

Instructed worry: As per Study Two (Chapter Three), the outcome measures in the mood/ state anxiety induction characterise changes resulting from the instructed worry phase. The measures are differences in VAS scores before and after instructed worry and number of thought intrusions (both general intrusions and negative intrusions) before and after instructed worry.

To assess the effect of mood/ state anxiety induction on VAS emotion scores, an rm-ANOVA was carried out on the VAS emotion scores with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and worry (pre-worry, post-worry) as within-subject variables. As expected, there was a significant main effect of worry $F(1, 62)= 8.218$, $p= .006$, $\eta_p^2= .117$, indicating that VAS emotion scores changed as a result of the mood/ state anxiety induction. As additionally expected, there was a statistically significant main effect of emotion $F(2.7, 169.1)= 41.992$, $p< .001$, $\eta_p^2= .404$), indicating that specific VAS

emotion scores varied systematically across emotions. The two way time x emotion interaction was also significant $F(2.7, 169.1)= 41.992$, $p< .001$, $\eta_p^2= .404$) (see Fig. 5.3.4.5.), reflecting post-worry increases in sadness and anxiety and reductions in happiness and calmness. However, there were no significant interactions with ABM or tDCS (all $p > .1$), indicating that VAS mood ratings in response to worry were not affected by the combination of tDCS and ABM.

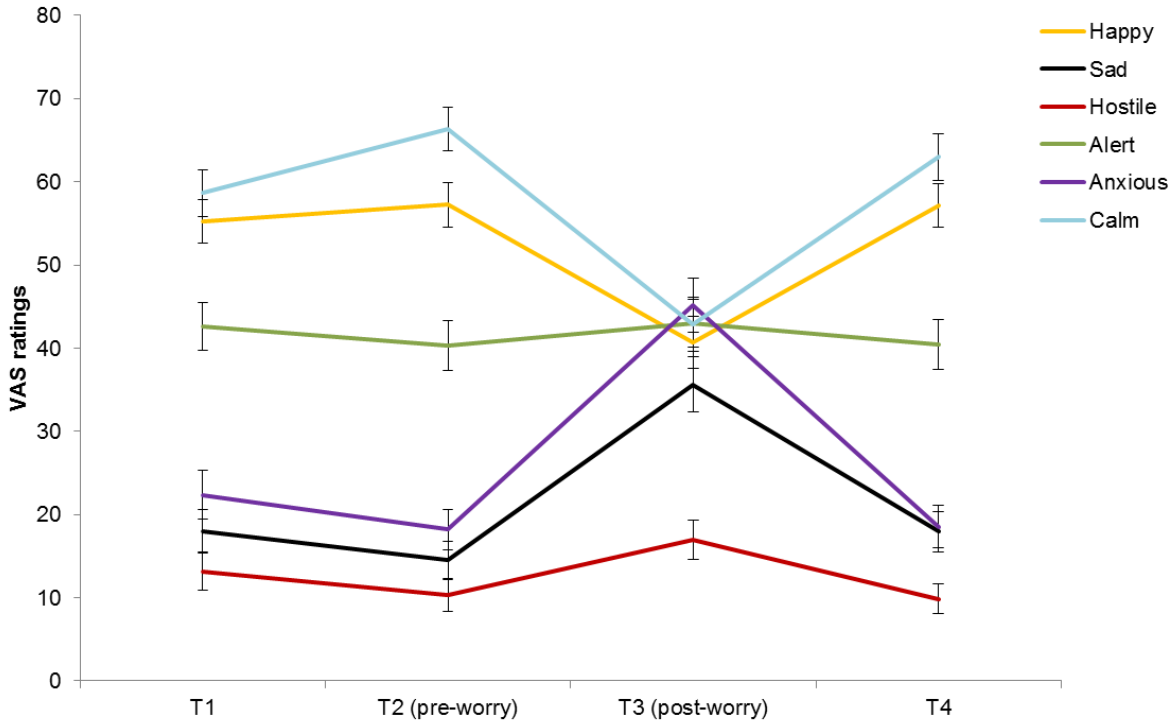

Figure 5.3.4.5. Changes in visual analogue scale (VAS) scores for different emotions over the course of the instructed worry task. T1: before task, T2: after first breathing focus, T3: after instructed worry period, T4: after second breathing focus. Scores are collapsed across tDCS/ABM conditions.

To assess the effect of state anxiety induction on thought intrusions, an rm-ANOVA was carried out on number of intrusions (positive and negative) with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. As expected, there was a statistically significant main effect of worry $F(1, 134)= 22.597$, $p< .001$, $\eta_p^2= .144$, reflecting a higher

number of intrusions after mood induction (M= 5.02, SD= 3.10) than before mood induction (M= 4.01 , SD= 2.63). There were no interactions with tDCS or ABM (all p>.06).

As negative thought intrusions were of particular interest, the analysis was repeated for the negative thought intrusions only (defined as rated below 5). An rm-ANOVA was carried out on number of intrusions with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and time (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically significant main effect of time $F(1,108)= 16.007$, $p< .001$, $\eta_p^2= .129$, reflecting a higher number of negative intrusions after instructed worry (M= 2.07, SD= 2.01) than before instructed worry (M= 1.27, SD= 1.89). However, there were no other main effects or interactions with ABM or tDCS (all p> .2).

Finally, the ratings (on a scale of 1-9, with 1 representing most negative and 9 representing most positive) of intrusions were calculated and the analysis was repeated for the ratings scores of the intrusions. An rm-ANOVA was carried out on mean intrusion ratings with tDCS (active, sham) and ABM (neutral, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically significant main effect of worry $F(1,123)= 8.896$, $p= .003$. $\eta_p^2= .067$, reflecting more negatively rated intrusions after instructed worry (M= 5.13, SD= 1.59) than before instructed worry (M= 5.67, SD= 1.59). However, there were no other main effects or interactions with ABM or tDCS (all p> .3).

5.3.5. Blinding

At the end of the study, participants were asked to indicate if they thought that they had received active or sham tDCS with a forced choice questionnaire. Twenty-two out of fifty-one (43.1%; 95%=C.-I.: 29.4 to 57.8%) of respondents correctly guessed their stimulation condition. Of the participants receiving sham tDCS, 8/27 (29.6%; 95%-C.-I.: 13.8 to 50.2%) of respondents correctly guessed their stimulation condition. Of the participants receiving active tDCS, 14/24 (58.3%; 95%-C.-I.: 36.6 to 77.9%) of respondents correctly guessed their stimulation condition. All exact binomial confidence-intervals included 50%, i.e. were not significantly different from chance.

5.4. Discussion

In a replication of the null findings in Study Two, the measures used in this study failed to reveal any clear statistical interactions between tDCS and ABM training on attentional bias and mood. Again, this is contrary to Clarke et al.'s (179) finding that anodal tDCS leads to greater evidence of attentional bias modification, using the same ABM training. Again, evidence from the sham tDCS group in the present study indicates that no basic training effect was observed. In addition, when examining subgroups that had a baseline attentional bias, there was still no effect of ABM. It is worth reiterating the that the cognitive tasks used in ABM do not always successfully modify attentional biases (225, 226) and that Clarke et al. did not find a basic training effect in their sham tDCS condition, although this was achieved with their tDCS group. Again, it would appear that the improvement of bias modification procedures in order to increase the reliability of their effects is key to the advancement of the field. However, there are some remaining design differences between the present study and Clarke et al., which merit further discussion.

The Clarke et al. study used a different electrode montage to the present study. The present design maintained the bipolar balanced DLPFC stimulation montage, as informed by the parameter optimisation in Study One instead of Clarke et al.'s unipolar montage with an extra cephalic reference (cathodal) electrode on the neck. Both montages stimulate the left DLPFC with the anodal electrode. However, Study Two and the present study also stimulated the right DLPFC with the cathodal electrode. Again, the difference in electrical field and current density/directionality from these two montages could produce different effects on cortical excitability at locations distal from the electrode location. In addition, Clarke et al. used 1mA current strength in their study, whilst the present study used 2mA based on a review of the depression literature. The variability of effects of tDCS at different current strengths is still under evaluation, with a recent study indicating that 20 mins of 2mA cathodal motor cortex stimulation actually had excitatory effects on motor evoked potentials, whereas 20 mins of 1mA cathodal stimulation had inhibitory effects (201). However, the overarching programme of the current research necessitated that 2mA of current was used in this study, in order to keep the current investigation consistent with parameters in most recent clinical trials (156). Nonetheless, it is possible that the differences between Clarke et al.'s findings and the present study are due to the electrode montage and current strength used.

The inclusion of a true control condition (with no bias modification) and baseline measurements did not improve the ability to detect an effect of tDCS and ABM. In fact, the inclusion of a baseline condition may have resulted in habituation to the task stimuli and possibly created a floor effect on vigilance, which ABM and tDCS could not alter. Additionally, neuroimaging research indicates that sensitivity to fearful faces is blunted after around 20 trials (228). The significant main effect of time on fear vigilance scores in

the faces task (particularly in the baseline vigilant sub groups) suggests that this is an important factor to consider in designing future studies as fear vigilance appears to be reduced over time generally. This may also explain the failure to replicate the vigilance reduction in the faces-dot probe task induced by DLPFC tDCS in Study One.

Indeed, it is possible that the ABM training intervention interfered with the reduction in vigilance previously observed (in Study One) when tDCS was given at rest. Aside from a recent pilot study indicating that concurrent cognitive control training may augment its antidepressant efficacy (229), clinical investigations of tDCS for mood disorders have traditionally focused on delivering stimulation at rest. Perhaps the 'at rest' factor is important to the clinical effects observed. Theoretically this could be explored by considering the potential interactions between tDCS and concurrent neural activity. For example, when a participant is undertaking a task that engages the DLPFC, it is more likely that DLPFC neurons will be depolarising. There is a chance that applying very weak direct current to already depolarising neurons will have little effect on their excitability, as membrane potential will already be fluctuating in this active state. On the other hand, applying weak electrical current to 'resting' DLPFC neurons could potentially induce resting membrane potential changes that may result in excitability modulation. This speculation could be examined practically by comparing behavioural effects of tDCS given at rest to tDCS given during a training paradigm. This comparison shall be made in a subsequent pooled analysis chapter (Chapter Six), utilising data from the first four experimental chapters of this thesis.

It is worth noting that subjects in the sham tDCS condition had a significant reduction in anxiety scores the day after testing and at two weeks after testing which was

not observed to the same extent in the active tDCS condition (collapsed across training conditions). This suggests that tDCS may have an anxiogenic effect, rather than the proposed anxiolytic effect which emerged from Study One. This may be a power issue which will be addressed in the pooled analysis chapter but is also worth monitoring in future studies, particularly with high anxious or clinical groups.

The experimental setup in the present study did not allow for double blinding, which is a potential limitation. However, forced choice questionnaire revealed that the blind was successful, with correct guesses of stimulation condition below chance.

Finally, in terms of information yield from Study Two and the present study, the instructed worry task was usefully modified to be delivered autonomously. This instructed worry task was a computerised version of a task that otherwise required two experimenters to execute (222). Although there were no effects of tDCS or ABM on performance in this task, its effect as a lab based mood/ state anxiety induction in healthy volunteers was replicated in the present study and could be usefully employed in future studies of worry and anxiety.

Chapter Six

Pooled data analysis

6.1. Introduction

The repetition of the Attentional Bias Modification (ABM) intervention across Study Two and Study Three, as well as the dot probe assessments, instructed worry and follow up questionnaires allow pooled data analysis to be carried out with increased statistical power. This allows the previously found null effects to be explored with greater confidence of avoiding type II errors and also allows specific questions to be addressed such as the comparison of three ABM conditions (attend threat, neutral, avoid threat). In addition, the inclusion of data from Study One in this pooled analysis allows a comparison of the effects of tDCS at rest and during ABM to be carried out without the need to collect further data.

6.2. Methods and materials:

Data from Study One (Chapter Two), Study Two (Chapter Three) and Study Three (Chapter Five) were used in this pooled analysis, with no further modifications. The bipolar unbalanced montage active condition was excluded from Study One as it was not comparable to the subsequent investigations. This resulted in the following sample sizes (some duplication across groups, e.g. baseline group subjects are also in other groups):

Sham tDCS at rest (Study One), n= 19

Active tDCS at rest (Study One), n= 20

Sham tDCS and neutral ABM (Study Three), n=16

Active tDCS and neutral ABM (Study Three), n=17

Sham tDCS and attend threat ABM (Study Two), n=20

Active tDCS and attend threat ABM (Study Two), n=20

Sham tDCS and avoid threat ABM (Study Two + Study Three), n=39

Active tDCS and avoid threat ABM (Study Two + Study Three), n=37

Baseline measurements (same participants, Study Three), n=69

Total N= 188

6.3. Results:

Pooled results are presented first for the two tDCS/ABM studies (Study Two and Study Three) and subsequently for all three tDCS studies (Study One, Study Two and Study Three).

6.3.1. Questionnaire based measurements – Study Two / Study Three

Acute VAS change: To assess acute emotion rating changes following tDCS and ABM rm-ANOVA was carried out on the VAS emotion ratings collected in Study Two and Study Three with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and time (pre, post) as within-subject variables. There were statistically significant main effects of time $F(1, 131)= 37.849$, $p< .001$, $\eta_p^2= .224$ and emotion $F(2.9, 392)= 404.859$, $p< .001$, $\eta_p^2= .756$ and the time x emotion interaction was also significant $F(3.3, 392)= 11.673$, $p< .001$, $\eta_p^2= .082$, indicating that specific emotion ratings varied systematically over time. The study x emotion interaction was also significant $F(2.9, 392)= 18.290$, $p< .001$, $\eta_p^2= .756$, indicating that emotion ratings varied systematically

across studies. There was also a trend emotion x tDCS interaction F(2.9, 392)= 2.524, p= .057, η_p^2= .019, reflecting a trend to different VAS ratings pre and post tDCS/ABM in the two tDCS conditions. However, there was no three-way interaction with time indicating that tDCS did not change the VAS scores from baseline in this pooled dataset. There were also no significant main effects or interactions with ABM (all p>.1), indicating that ABM did not affect emotion ratings acutely in this pooled dataset.

6.3.2. Questionnaire measurements (follow up) – Study Two / Study Three

Follow up data were also collected in Study Two and Study Three. There were only moderate levels of compliance on follow up questionnaires and resulting loss of data for both studies. Therefore the sample size to be analysed at each follow up period dropped from 134 (baseline) to 117 (day 2), 110 (day 14) and 117 (day 28).

State/Trait anxiety follow up: State anxiety scores (STAI-S) were recorded at baseline, the day after testing and at 2 weeks follow up and trait anxiety scores (STAI-T) were recorded at baseline and at 4 weeks after testing. State/ trait anxiety scores were analysed using rm-ANOVA; with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and time ((baseline, day 28 for STAI-T) or (baseline, day 2, day 14 for STAI-S)) as a within-subject variable. There was a significant main effect of time on trait anxiety scores F(1, 100)= 11.360, p= .001, η_p^2= .102, a significant main effect of study F(1, 100)= 103.614, p< .001, η_p^2= .509, and the time x study interaction showed a trend F(1, 100)= 3.657, p= .059, η_p^2= .035, There was also a significant main effect of time on state anxiety scores F(1.9, 157.5)= 9.742, p< .001, η_p^2= .105, a significant main effect of study F(1, 83)= 12.645, p= .001, η_p^2= .132 and a significant main effect of tDCS F(1, 83)= 7.624, p= .007, η_p^2= .061. However, there were

no interactions between time and tDCS or ABM on trait or state anxiety scores (all p>.3) indicating that state anxiety was not modified as a result of tDCS/ABM in this pooled dataset.

6.3.3. Behavioural measurements – Study Two / Study Three

Faces dot probe tasks: 'Vigilance to threat' was operationally defined as in Chapter Two. Face pairs were presented at two different durations, short (100ms) and medium (500ms), which were analysed independently. Analysis of short-duration trials aimed to test whether tDCS would abolish fear vigilance that is characteristic of rapid responses to threat seen in anxiety disorders. Analysis of medium-duration trials tested whether tDCS would abolish fear vigilance on a timescale similar to that of the training task.

Short duration dot probe task (100ms): To assess the effects of tDCS and training on emotional vigilance at short durations, rm-ANOVA was carried out on the vigilance scores with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables, and emotion (happy, fearful) as a within-subject variable. There was a significant emotion x study interaction $F(1, 139)= 4.022$, $p= .047$, $\eta_p^2= .028$. However, there were no other significant main effects or interactions (all p> .1), reflecting similar vigilance for fearful faces and happy faces after tDCS/ABM, indicating that emotional vigilance at short durations was not modified by the combination of tDCS and ABM in this pooled dataset.

Medium duration dot probe task (500ms): To assess the effects of tDCS and ABM on emotional vigilance at medium durations, rm-ANOVA was carried out on the vigilance scores with study (two, three), tDCS (active, sham) and ABM (neutral, attend threat, avoid

threat) as between-subjects variables, and emotion (happy, fearful) as a within-subject variable. Again, there was a significant emotion x study interaction F(1, 139)= 3.932, p= .049, η_p^2= .028. However, there were no significant main effects or interactions (all p> .09), reflecting similar vigilance for fearful faces and happy faces after tDCS/ABM, indicating that emotional vigilance at medium durations was not modified by the combination of tDCS and ABM in this pooled dataset.

Dot probe words: 'Vigilance to threat' was operationally defined as in Chapter Three. To assess the effect of tDCS and ABM, ANOVA was carried out on the 'vigilance to threat' scores with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as fixed factors. There were no significant main effects or interactions (all p>.2), reflecting similar vigilance for threatening and neutral words after tDCS/ABM, indicating that emotional vigilance to threatening words was not modified by the combination of tDCS and ABM in this pooled dataset. To test for a training effect of ABM, the analysis was repeated using only the sham tDCS group. There was no main effect of ABM F(2,70)= .703, p= .499 meaning that vigilance to threatening words was not affected by the type of ABM received in this pooled dataset.

Instructed worry: As per Study Two (Chapter Three), the outcome measures in the state anxiety induction characterise changes resulting from the instructed worry phase. The measures are differences in VAS scores before and after instructed worry and number of intrusions (both general intrusions and negative intrusions) before and after instructed worry.

To assess the effect of state anxiety induction on VAS emotion scores, an rm-ANOVA was carried out on the VAS emotion scores with study (two, three), tDCS (active,

sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and worry (pre-worry, post-worry) as within-subject variables. As expected, there was a significant main effect of worry $F(1, 135)= 13.785$, $p< .001$, $\eta_p^2= .093$, indicating that VAS emotion scores changed as a result of the mood/ state anxiety induction. As additionally expected, there was a statistically significant main effect of emotion $F(2.9, 400.6)= 214.602$, $p< .001$, $\eta_p^2= .614$. The interaction of emotion x worry was also significant $F(2.8, 381.8)= 101.325$, $p< .001$, $\eta_p^2= .429$, indicating that specific VAS emotion scores varied systematically across specific emotions (see Fig. 6.3.3.1.), replicating the effects observed in Study Two and Study Three.

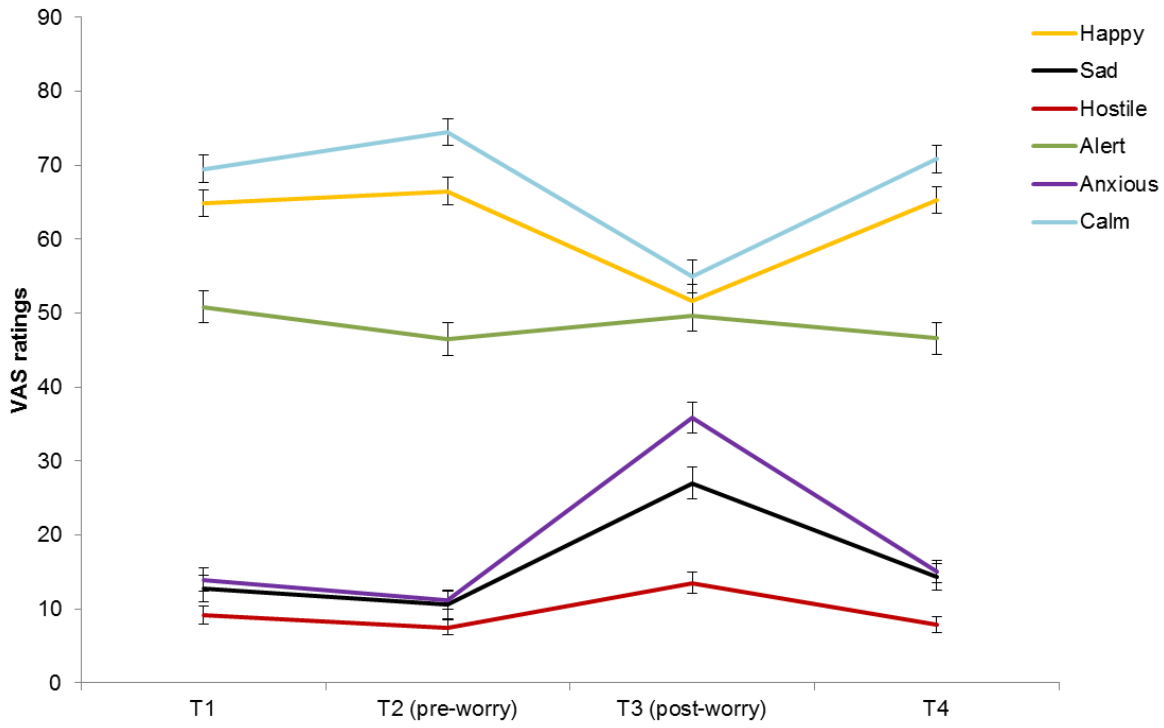

Figure 6.3.3.1. Changes in visual analogue scale (VAS) scores for different emotions over the course of the instructed worry task. T1: before task, T2: after first breathing focus, T3: after instructed worry period, T4: after second breathing focus. Scores are collapsed across tDCS/ABM conditions.

The two-way worry x ABM interaction was significant $F(2, 135)= 3.810$, $p= .025$, $\eta_p^2= .053$, and the two-way emotion x study interaction was also significant $F(2.9, 400.7)=$

15.875, p< .001, η_p^2= .105. The two-way interaction of ABM with worry indicated that emotion rating changes post worry may depend on ABM condition. However, the three way worry x emotion x ABM interaction was not significant F(5.7, 381.8)= .573, p= .742, indicating that ABM did not modify the effect of worry on specific emotions.

To further investigate the significant two-way interactions with ABM, post-hoc rm-ANOVAs were carried out on the VAS emotion scores, separately for each emotion with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. There was a significant worry x tDCS interaction F(1, 135)= 8.668, p=.004, η_p^2=.060 on alertness scores reflecting an increase in alertness post worry in the active tDCS condition. However, this could be caused by differences in baseline alertness between the tDCS conditions (see Fig. 6.3.3.2.). There were no other interactions with tDCS or ABM on specific VAS emotion change scores in this pooled dataset.

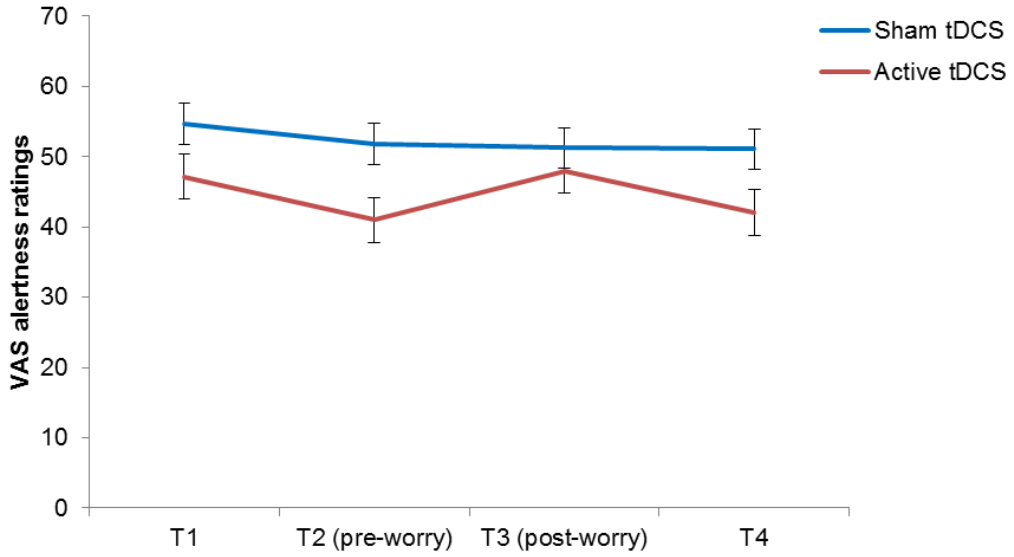

Figure 6.3.3.2. Effect of tDCS on worry induced alertness ratings. Values denote mean VAS ratings (before and after worry). Scores are collapsed across ABM conditions. Error bars represent +- 1 standard error of the mean.

To assess the effect of mood/ state anxiety induction on thought intrusions, rm-ANOVA was carried out on number of intrusions (positive and negative collapsed) with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. As expected, there was a statistically significant main effect of worry $F(1, 132)= 24.676$, $p< .001$, $\eta_p^2= .157$, reflecting a higher number of intrusions after mood/ state anxiety induction ($M= 5.02$, $SD= 3.09$) than before mood/ state anxiety induction ($M= 4.01$, $SD= 2.63$). . However, there were no interactions with tDCS or ABM (all $p> .06$).

As negative thought intrusions were of particular interest the analysis was repeated for the negative thought intrusions only (defined as rated below 5 on a scale of 1-9, with 1 representing most negative and 9 representing most positive). An rm-ANOVA was carried out on number of intrusions with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and time (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically significant main effect of time $F(1, 106)= 19.576$, $p< .001$, $\eta_p^2= .156$, reflecting a higher number of negative intrusions after instructed worry ($M= 2.08$, $SD= 2.01$) than before instructed worry ($M= 1.27$, $SD= 1.90$). However, there were no other main effects or interactions with ABM or tDCS (all $p> .06$).

Finally, the valence ratings (on a scale of 1-9, with 1 representing most negative and 9 representing most positive) of intrusions were calculated and the analysis was repeated for the mean valence rating scores of the intrusions. An rm-ANOVA was carried out on the mean intrusion ratings with study (two, three), tDCS (active, sham) and ABM (attend threat, neutral, avoid threat) as between-subjects variables and worry (pre-worry, post-worry) as a within-subject variable. Again, as expected, there was a statistically

significant main effect of worry F(1, 121)= 9.839, p= .002, η_p^2= .075, reflecting more negatively rated intrusions after instructed worry (M= 5.14, SD= 1.47) than before instructed worry (M= 5.67, SD= 1.59). There were no other main effects or interactions with ABM or tDCS (all p> .1).

6.3.4. Questionnaire based measurements – Study One / Study Two / Study Three

Analysis of the data collected from Study One, Study Two and Study Three together allows a larger sample to compare the effects of real and sham tDCS (collapsed across ABM conditions) and also, to compare the effects of tDCS delivered at rest (Study One) with tDCS delivered during an ABM intervention (Study Two/ Study Three).

Acute VAS change: To assess acute emotion rating changes following active tDCS compared to sham tDCS (collapsed across ABM conditions) rm-ANOVA was carried out on the VAS emotion ratings collected in Study One, Study Two and Study Three with study (one, two, three) and tDCS (active, sham) as a between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and time (pre, post) as within-subject variables. There were statistically significant main effects of time F(1, 168)= 23.509, p< .001, η_p^2= .123 and emotion F(2.8, 473.7)= 505.592, p< .001, η_p^2= .751, indicating that VAS ratings varied over emotion types, after active or sham tDCS. There was also a significant two-way time x emotion interaction F(3.2, 544.3)= 9.29, p< .001, η_p^2= .052, reflecting decreases in alertness and calmness after active or sham tDCS. In addition, there was a significant three way time x emotion x study interaction F(6.5, 544.3)= 2.49, p= .019, η_p^2= .029. However, there were no main effects or interactions with tDCS (all p>.08) indicating that there was no difference in VAS score changes after active compared to sham tDCS.

Next, to assess acute emotion rating changes following tDCS received at rest (without ABM) compared to tDCS received during avoid threat ABM (the active ABM condition); rm-ANOVA was carried out on the VAS emotion change scores collected from the active tDCS conditions in Study One, Study Two and Study Three with study (one, two, three) and ABM (none, avoid threat) as a between-subjects variables and emotion (happy, sad, hostile, alert, anxious, calm) and time (pre, post) as within-subject variables. There were statistically significant main effects of time $F(1, 48)= 7.320$, $p= .009$, $\eta_p^2= .132$ and emotion $F(3.3, 159.8)= 131.673$, $p< .001$, $\eta_p^2= .733$, indicating that VAS ratings varied over different emotions and also after tDCS (with or without ABM), although the lack of a two-way interaction $F(2.8, 138.9)= 1.658$, $p= .181$, means this was not systematic for specific emotions. There was a significant emotion x study interaction $F(3.3, 159.8)= 8.439$, $p< .001$, $\eta_p^2= .150$, indicating that emotion rating varied across studies. In addition, there were no main effects or interactions with ABM (all $p> .2$) indicating that there was no difference in VAS score changes after tDCS delivered at rest and tDCS delivered with ABM.

6.3.5. Behavioural measurements – Study One / Study Two / Study Three

To assess the effect of tDCS on subsequent vigilance to threat at short durations in the faces dot probe, vigilance data was pooled across Study One, Study Two and Study Three. To assess acute emotion rating changes following active tDCS compared to sham tDCS (collapsed across ABM conditions) rm-ANOVA was carried out on the vigilance scores with study (one, two, three) and tDCS (active, sham) as a between-subjects variables and emotion (happy, fear) as a within-subject variable. There was a statistically significant main effect of emotion $F(1, 180)= 5.031$, $p= .026$, $\eta_p^2= .027$, reflecting expected vigilance towards fearful faces ($M= 5.21$, $SD= 50.01$) and away from happy faces ($M= -5.19$, $SD=$

43.69) and the emotion x study interaction was also significant F(2, 180)= 3.242, p= .041, η_p^2= .035. However, the emotion x tDCS interaction was not significant F(1, 180)= 0.828, p= .364, and the emotion x study x tDCS interaction was also not significant F(2, 180)= 2.773, p= .065, indicating that vigilance was not affected by tDCS in this pooled dataset.

Next, to assess the effects of tDCS received at rest (without ABM) compared to tDCS received during avoid threat ABM (the active ABM condition) on subsequent vigilance to threat at short durations in the faces dot probe; rm-ANOVA was carried out on the vigilance scores in the active tDCS groups only with study (one, two, three) and ABM (none, avoid threat) as a between-subjects variables and emotion (fear, happy) as a within-subject variable. The main effects of study, emotion, ABM and the ABM x emotion interaction were not significant (all p>.1). This suggests that there is no difference between fear vigilance after tDCS delivered at rest and tDCS delivered at the same time as an ABM intervention.

The pooled data from the three experimental studies is summarised in Fig. 6.3.5.1., using the sub-groups described above.

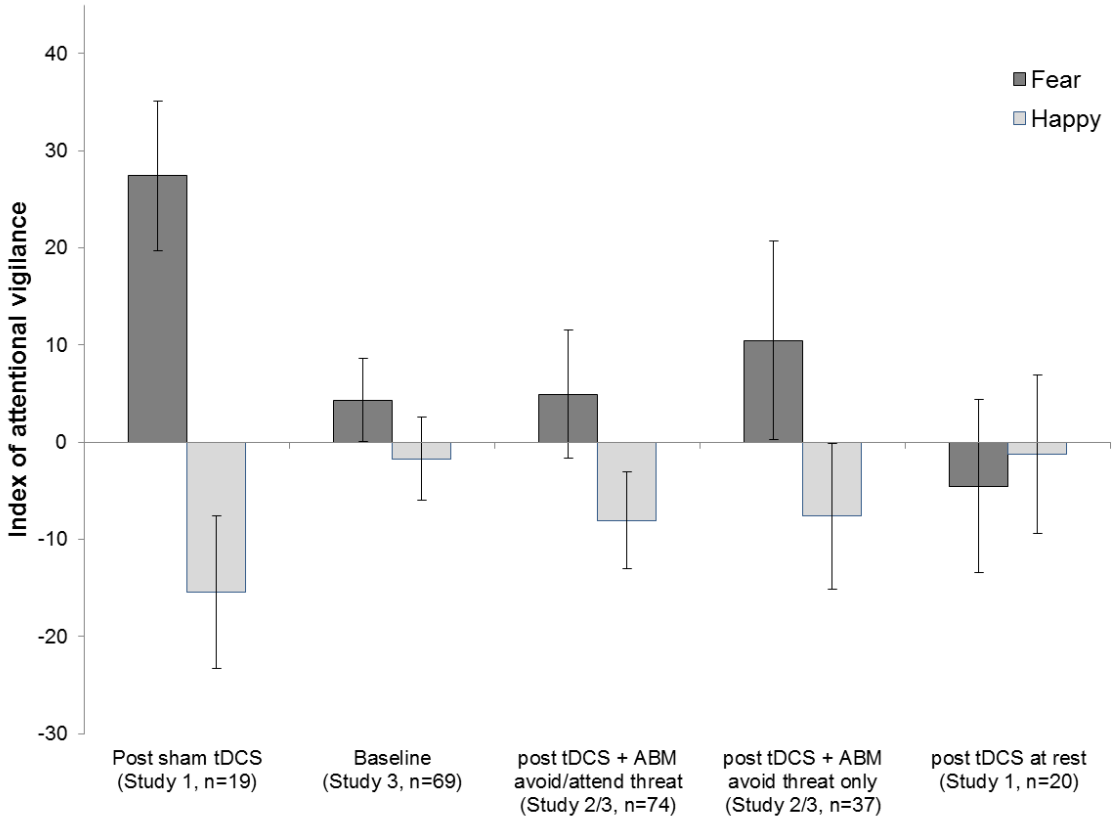

Figure 6.3.5.1. Dot probe emotional vigilance. Vigilance scores represent the difference between reaction times (ms) when the probe was located behind the emotional face versus when the probe was located behind the neutral face of the same pair type. Error bars represent +- 1 standard error of the mean.

6.4. Discussion

Pooling of data from three studies allowed statistical tests to be carried out with greater power to detect an effect, decreasing the likelihood of potential type II errors as well as providing some additional comparisons between groups that only existed in the separate studies. Some trends emerged from this pooled dataset, such as trend effects of tDCS on alertness and overall intrusions. The latter effect could be related to the proposed efficacy of tDCS in augmenting cognitive control (229, 247).

Overall these trends were weak with baseline differences, small effect sizes and this pooled analysis revealed no significant effects of tDCS or ABM on acute mood

changes, emotional vigilance or mood induction. This adds strength to the previous null findings but also raises questions regarding the robustness of the baseline vigilance phenomenon from Study One. There is a potential concern over the reliability of the dot probe measure because of differences that have emerged in baseline/sham vigilance when examining across studies. However, the baseline dot probe face measurements in Study Three were carried out after baseline dot probe word assessments and so the reduction in vigilance in the baseline dot probe faces measure could be an after effect of habituation to threatening stimuli. The findings from Study Three and the pooled analysis did reveal an effect of time on vigilance, with fear vigilance reliably reducing over time, regardless of tDCS/ABM intervention. This suggests a strong habituation effect to fearful stimuli which is supported by the neuroimaging literature (228). Nonetheless, the reliability of the dot probe measure is under question (248, 249), the findings from Study One require replication in a larger sample and future studies should incorporate alternative measures of vigilance.

Sensitive neuroimaging measurements of attentional control and threat vigilance are needed to probe the phenomenon put forward by Study One and to give confidence to the behavioural findings in the light of the uncertainty resulting from the ABM investigations in Study Two and Study Three. In this pooled analysis, the instructed worry intervention was again shown to have robust effects on mood ratings, although differences in the sample characteristics (healthy versus trait anxious) made a formal internal reliability analysis of this measure between Study Two and Study Three impossible.

Finally, although it is possible that the findings from Clarke et al. (179) were the result of the parameter differences (electrode size and current strength) between the two

designs, it was decided that because of the repeated failure to detect an effect of ABM, future tDCS investigations in this programme of work (including the fMRI study described in the next chapter) would not include an ABM component.

Chapter Seven

A causal role for dorsolateral prefrontal cortex in modulating amygdala response to threat

7.1. Introduction

Animal studies provide compelling evidence for the importance of the pre-frontal cortex (PFC) in regulating responses to threat via direct inhibition of the amygdala complex. The PFC acts to inhibit aversive associations established in fear conditioning, with PFC lesions impeding (72) and conversely, electrical stimulation of the PFC enhancing (73) the extinction of a conditioned response in mice and rats. Furthermore, electrical pre-stimulation of the PFC in rats and cats (76) specifically blocks or reduces subsequent response from amygdala output neurons. The results from these pre-clinical investigations provide the foundation for theoretical models of emotional dysfunction in disorders of depression and anxiety. As such, these disorders have been hypothesised to occur when there is a failure of cortical modulation of the lower-level limbic response to threat (77, 78). Consistent with this, these disorders are associated with hyperactive amygdala (66, 118) and/or hypoactive PFC (119); resulting in an imbalance in activity levels of this cortico-limbic circuit in neuroimaging studies, which has been shown to reverse with treatment (79-82). However, there is no direct evidence that this circuit functions in the same way in humans as reported in pre-clinical animal models, i.e. that frontal control regions, including the DLPFC, inhibit amygdala responses to threat.

The findings from Study One suggest that frontal cortex tDCS reduces vigilance to threat in a dot probe paradigm (250). The underlying neural mechanism for this is proposed to be increased regulation of amygdala threat response through improved top-down attentional control from the PFC. This proposed improvement in attentional control could be specific to fearful stimuli or more general. The ability to target the DLPFC with tDCS provides a unique experimental probe to explore the relationship between the response of the PFC and subcortical regions such the amygdala. By using tDCS to change the electrical state of cortical tissue, it becomes possible to test causal hypotheses about functional interactions between cortex and interconnected subcortical structures (251). The ability to combine tDCS with neuroimaging methods provides a powerful set of tools to explore the neural correlates of the observed behavioural phenomenon.

Therefore DLPFC tDCS was used to probe the causal impact of altered PFC function on blood oxygen level-dependent (BOLD) response in the amygdala and behavioural response in a high anxious subject group, using tasks from a well validated attentional control paradigm (85, 124). Using an emotional and non-emotional version of this attentional control paradigm also allows the question of emotional specificity to be addressed. Previous work showed that fearful faces elicit right amygdala response under conditions of low but not high cognitive effort in this paradigm, suggesting dependence on the availability of attentional capacity. Compared to low anxious subjects, high anxious subjects show hypoactive frontal and hyperactive amygdala responses to fearful faces in this paradigm, as well as impaired accuracy (85). This is thought to reflect poor attentional control in trait anxiety. We hypothesised that DLPFC tDCS could modulate this pattern of activation and behaviour.

7.2. Methods and Materials

7.2.1. Participants

Sixteen female participants (all right handed, aged 18-45 years) performed a visual search task adapted from Bishop and others (85, 124) while functional magnetic resonance imaging (fMRI) data were acquired. The study was approved by the Central University Research Ethics Committee (University of Oxford) and performed in compliance with their approved protocols for tDCS and MRI. Participants were pre-screened with an online version of the State-Trait Anxiety Inventory (STAI - (252)) and those who scored more than 45 on the trait anxiety questionnaire (STAI-T) were invited to a screening session at the Warneford Hospital, Headington. Participants STAI-T scores ranged from 45 to 63 (M= 53, SD= 5). After informed consent was obtained, participants were screened using the structured clinical interview for DSM-IV disorders and based on this individuals with current depressive episode, current or past neurological disease or family history of bipolar disorder were excluded, as were individuals on medication for an anxiety disorder or depression or with any contraindications to MRI or tDCS. Participants were compensated for their time at a rate of £10 per hour.

7.2.2. Design

This study used a within-subjects, double-blind design with 16 participants randomised to a stimulation order condition (active/sham tDCS followed by sham/active tDCS one month later, counterbalanced). On the day of the study participants filled out some mood questionnaires before being introduced to the scanner environment and receiving a structural scan while they practiced the attentional control task. Then they vacated the scanner and received tDCS in a separate room while they sat at rest. This allowed the participant to practice the task and become comfortable in the scanner environment to

minimise time spent entering the scanner after tDCS. After the stimulation ended the participants were reintroduced into the scanner (with a mean time from tDCS to task of ~7 minutes) and carried out the attentional control task while fMRI data were acquired.

7.2.3. Attentional control tasks

Visual stimuli were back projected onto a translucent screen positioned behind the bore of the magnet, visible via an angled mirror placed above the participant's head. The letter search task used (and following description) was taken from Bishop and others (85), adapted from Jenkins and others (253). Two versions of the letter search task were used, one with facial distractors and the other with letter distractors. For the face distractor task, on each trial, a string of 6 letters superimposed on a task-irrelevant non-familiar face, was presented for 200 ms (see Fig. 7.2.3.1.a.). In the present study the face stimuli comprised four different individuals with fearful and neutral expressions taken from the Pictures of Facial Affect (254) and cropped to remove extraneous background information. The neutral faces used were morphed using computer graphics to 30% happy as neutral faces have been previously found to be aversive. For the letter distractor task, on each trial, a string of 6 letters accompanied by a distractor letter, was presented for 200 ms (see Fig. 7.2.3.1.b.). The distractor letter was slightly larger than the letters in the letter string and could appear above or below the string. The distractor letter was either congruent (the same as the target, either "X" or "N"), incongruent (the same as the other target, either "X" or "N") or neutral (the letter "C").

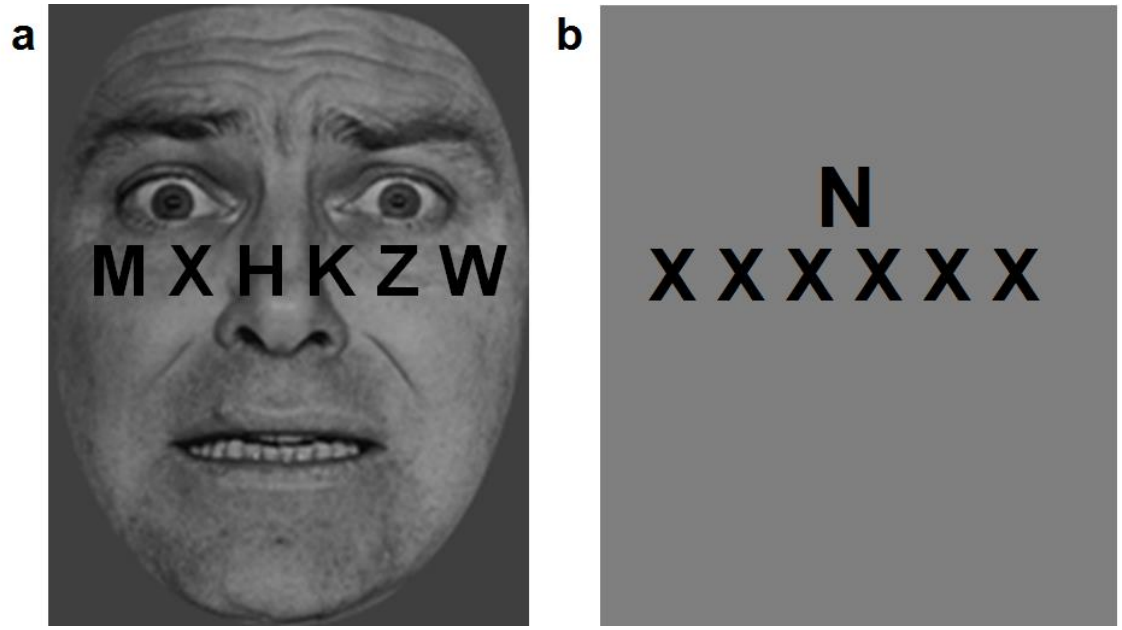

Figure 7.2.3.1. Example stimuli. (a) On each trial, a string of 6 letters was superimposed on a face presented in the centre of the screen. Participants had to indicate whether the letter string contained an "X" or an "N", a target always being present (face stimuli reproduced with permission from Ekman and Friesen 1976) (b) On each trial, a string of 6 letters was accompanied by a distractor letter, either above or below the letter string. Participants had to indicate whether the letter string contained an "X" or an "N", a target always being present

There were 3 interleaved imaging acquisition runs of each type of task (6 imaging runs in total), each comprised 12 blocks of 4 trials. Participants were instructed to indicate with a keypress whether the letter string contained an 'X' or an 'N'. In half the blocks for each task – the *'high cognitive effort'* condition – the string comprised a single target letter (N or X) and 5 non-target letters (H, K, M, W, Z) arranged in random order. In the other half of the blocks—the 'low cognitive effort' condition—the letter string comprised 6 Xs or 6 Ns, reducing attentional search requirements. This manipulation of cognitive effort is identical to the one used in Bishop and others (85) Jenkins and others (253) and conforms to Lavie's description of heightening cognitive effort by 1) increasing the number of different identity items that need to be perceived or 2) making perceptual identification more demanding on attention (127, 253).

There was a 2 s interval between blocks. Within blocks, the interstimulus interval was randomly jittered using an exponential function with a mean of 4.5 s and a minimum of 3 s. A mixed block/event-related design was used—the level of cognitive effort for the letter search task (high or low) being varied across blocks, the expression of the faces (fearful or neutral) or congruency of the distractor (congruent, incongruent or neutral) being varied within blocks on a trial by trial basis. The 2 factors in the faces distractor task resulted in 4 conditions of interest for the faces task:

(i) high load, fearful distractor

(ii) high load, neutral distractor

(iii) low load, fearful distractor

(iv) low load, neutral distractor

The 3 factors in the letter distractor task resulted in 6 conditions of interest for the letter task:

(i) high load, congruent distractor

(ii) high load, incongruent distractor

(iii) high load, neutral distractor

(iv) low load, congruent distractor

(v) low load, incongruent distractor

(vi) low load, neutral distractor

By examining the effect of tDCS upon the neural response to fearful versus neutral face distractors under conditions of low load, it was possible to test the hypothesis that tDCS reduces vigilance to threat through effects on fronto-limbic activity relevant to attentional control. Additionally, examining the effect of tDCS upon the neural response

to incongruent versus congruent distractors under conditions of low load, it was possible to test the hypothesis that tDCS increases attentional control more generally in a trait anxious group.

7.2.4. Transcranial Direct Current Stimulation (tDCS)

Stimulation was delivered as per Study One (Chapter Two). After participants were familiarised with the MRI scanning environment they were taken to a separate room and stimulation was applied while the participant sat at rest. After stimulation the participant was reintroduced to the scanner to complete the computerised tasks.

7.2.5. Image Acquisition

Blood oxygenation level dependent (BOLD) contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens Magnetom TrioTim syngo MRB17 with a head coil gradient set. Each image was made up of 45 interleaved 3mm thick slices, interslice gap 1mm; field of view 25x25cm; matrix size 64 x 64; flip angle 87° echo time (TE) 30ms; voxel bandwidth 2368 Hz/Px; acquisition time (TA) 2.3 s; repetition time (TR), 2710ms. Slice acquisition was interleaved and covering the whole brain with an additional z shim to reduce distortion in the orbitofrontal cortex. Data were acquired in 6 scanning runs of 5 min. The first 5 volumes of each run were discarded to allow for T1 equilibration effects.

7.2.6. FMRI data analysis

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB Software Library, www.fmrib.ax.ac.uk/fsl). Registration to high resolution structural and standard space was carried out using FLIRT (255, 256).

Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear registration (257, 258). An intermediate analysis was then carried out, combining three runs of each task into a single dataset for each participant (16) for each task (2) for each testing session (2). Finally, a within-subjects analysis was performed and Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of p=0.05. Region of interest (ROI) analyses were carried out using small volume corrections for the amygdala region of interest. Amygdala ROIs were defined using the Harvard-Oxford Cortical Structural Atlas (including all voxels with a greater than 50% probability of lying within the amygdala). All activations are reported using voxel co-ordinates.

7.3. Results

The response amplitudes to unattended fearful and neutral faces or congruent/incongruent/neutral distractor letters (under conditions of high and low cognitive effort) were characterised on the basis of evoked BOLD responses and behavioural scores (reaction time and accuracy). Contrasting the effects of fearful and neutral distractors in an attentional control paradigm allows a quantification of the effects of fear on attentional control, which is thought to be impaired as a feature of trait anxiety. In addition, a comparison of the effects of active versus sham tDCS on neural and behavioural responses to fear allows a quantification of the effects of tDCS on fear processing in anxious subjects. Contrasting the effects of congruent and incongruent or neutral distractors in an attentional an attentional control paradigm allows a quantification of the effects of distractor congruence on attentional control, which is thought to be impaired as a feature of trait anxiety. In addition, a comparison of the effects of active versus sham tDCS on neural and behavioural responses to distractors allows a

quantification of the effects of tDCS on attentional control more generally in anxious subjects.

7.3.1. Neuroimaging results

Face distractor task: Consistent with previous work, we showed baseline right amygdala activation in response to threat under conditions of low cognitive effort (svc in sham treated participants, p<.05) (Fig. 7.3.1.1), meaning that, as expected, the right amygdala was reactive to fearful faces during low cognitive effort only.

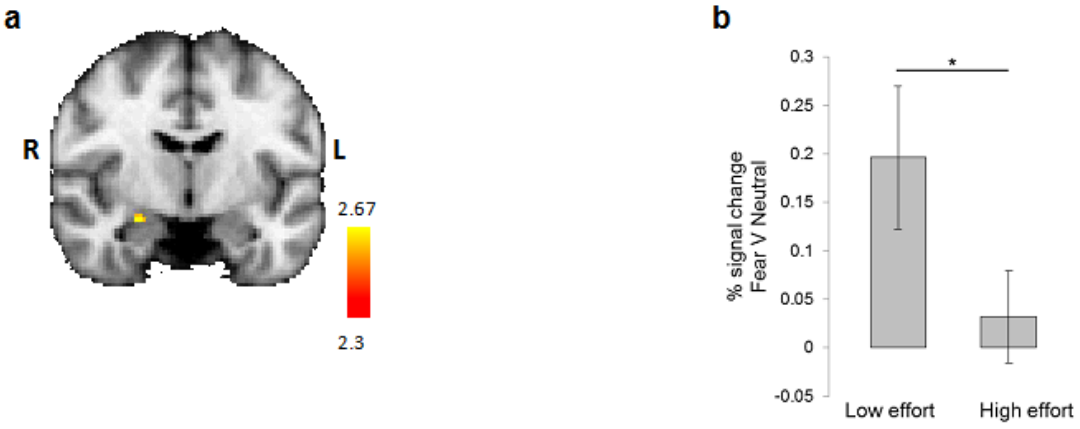

Figure 7.3.1.4. Effect of fearful faces on amygdala response under low versus high cognitive effort. (a) Thresholded statistical map depicts the cluster in the right amygdala (p<.05, svc) for fearful face distractors versus neutral face distractors under conditions of low cognitive effort compared to conditions of high cognitive effort. (b) Right amygdala mean percent signal change for the significant cluster from (a) for sham tDCS, fearful versus neutral face distractors against cognitive effort (low, high). The functional maps are overlaid on an average anatomical image spatially normalised to MNI space. Error bars represent +-1 standard error of the mean (SEM).

This baseline amygdala response was causally modified by tDCS. Direct current stimulation over bilateral DLPFC led to a reduction in bilateral amygdala (svc, p<.05) (Fig. 7.3.1.2) response to fearful facial expressions during low cognitive effort.

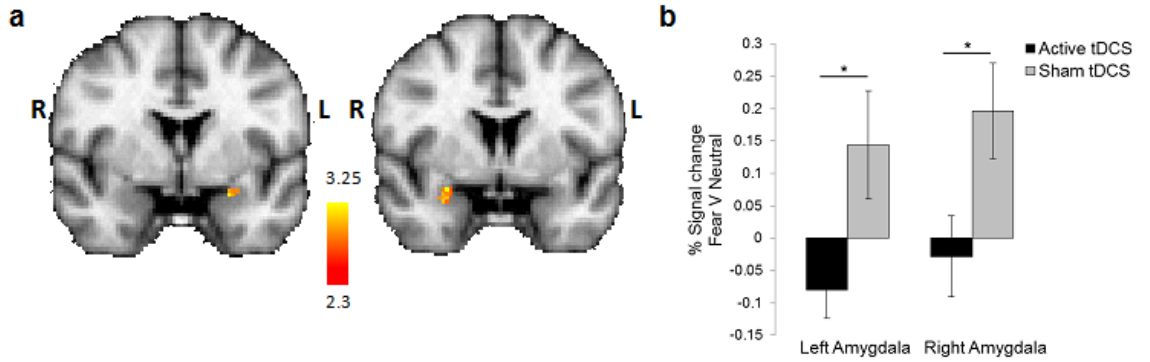

Figure 7.3.1.2. Effect of tDCS on amygdala response to fearful faces. (a) Thresholded statistical map depicts the clusters in the right and left amygdala (p<.05, svc) for fearful face distractors versus neutral face distractors under conditions of low cognitive effort. Amygdala ROIs defined using the Harvard-Oxford Cortical Structural atlas (thresholded at 50% probability). (b) Right and left amygdala mean percent signal change for the significant cluster from (a) for sham versus active tDCS, fearful versus neutral face distractors under conditions of low cognitive effort. The functional maps are overlaid on an average anatomical image spatially normalised to MNI space. Error bars represent +-1 standard error of the mean (SEM).

Furthermore, stimulation led to an increase in neural response in the frontoparietal sites (Fig. 7.3.1.3) in the same trial type.

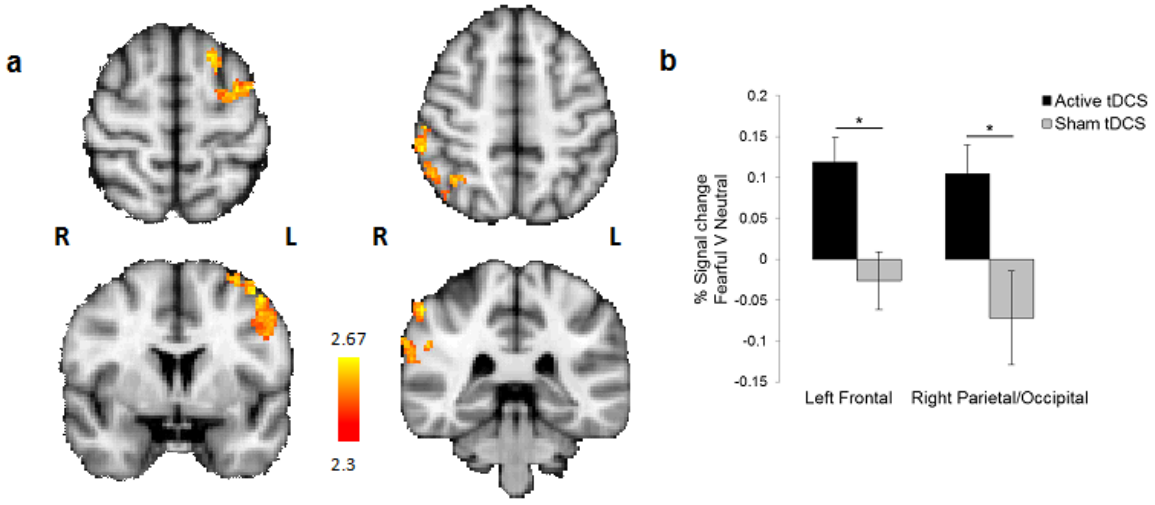

Figure 7.3.1.3. Effect of tDCS on frotoparietal response to fearful faces. (a) Thresholded statistical maps (P<0.05, corrected) depict, from right to left, significant clusters in Left frontal cortex and Right Parietal/Occipital cortex for active tDCS compared to sham tDCS to fearful face distractors versus neutral face distractors under conditions of low cognitive effort (b) Mean percent signal change for the significant clusters from (a) for active versus sham tDCS, fearful versus neutral face distractors under conditions of low cognitive effort. The functional maps are overlaid on an average anatomical image spatially normalised to MNI space. Error bars represent +-1 standard error of the mean (SEM).

Condition, contrast	Analysis	Cluster co-ordinates	Hemisphere	N sig voxels	Location	z-score max	Sig (p-corrected)
Sham only, (LF-LN)-(HF-HN)	SVC	34 59 28	Right	20	Right amygdala	3.06	.0356
Sham-active, LF-LN	SVC	31 64 28	Right	15	Right amygdala	3.295	.0397
Sham-active, LF-LN	SVC	57 63 27	Left	9	Left amygdala	2.816	.0401
Active-sham, LF-LN	Whole brain	16 45 61	Right	757	Right angular gyrus	3.47	.000564
Active-sham, LF-LN	Whole brain	68 65 62	Left	538	intraparietal sulcus, frontal eye fields	3.74	.00665

Table 7.3.1.1. Summary of a priori region (bilateral amygdala) and whole brain voxel wise analysis. Effects of cognitive load on fear response and effects of tDCS on fear response. LF= Low perceptual load, fearful face, LN= Low perceptual load, neutral face, HF= High perceptual load, fearful face, HN= High perceptual load, neutral face. SVC= Small volume correction.

Letter distractor task: Whole brain and ROI analysis did not reveal any effects of tDCS on neural response to distractor letters (incongruent vs congruent) in conditions of low or high cognitive effort. This suggests that tDCS does not affect neural activity relating to non-emotional attentional control.

7.3.2. Behavioural results

Face distractor task: For the fearful face distractor trials, participants in the sham session were slower to respond (39.2% slower; pair wise t-test: $t(15)= 6.934$, $p<. 001$) and less accurate in their responses (10.6% lower accuracy, pair wise t-test: $t(15)= 2.976$, $p= .009$) under conditions of high cognitive effort compared to conditions of low cognitive effort.

Following on from previous work showing reduced accuracy in high anxious subjects, DLPFC stimulation increased accuracy on this task for unattended fear face trials under low cognitive effort (9.1% higher accuracy, pair wise t-test: $t(15)= 1.936$, $p= .036$,

one tailed) compared with when they received sham stimulation (Fig. 7.3.1.4.). There was no effect of stimulation on reaction times.

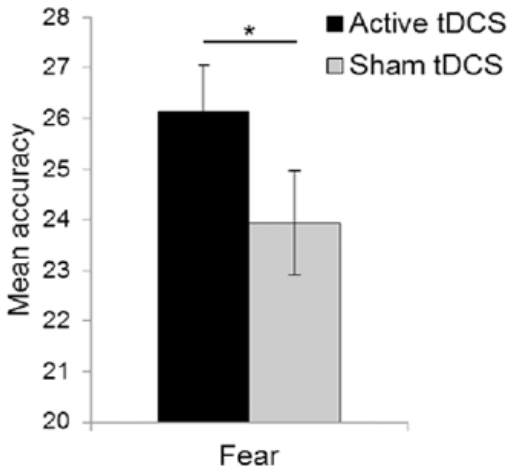

Figure 7.3.1.4. Mean accuracy in the attentional control task for active versus sham tDCS for trials with fearful face distractors under conditions of low cognitive effort. Error bars represent +-1 standard error of the mean (SEM).

Letter distractor task: Paired samples t-tests revealed that there were no significant effects of tDCS on accuracy or reaction times in the letter distractor task, under low or high cognitive effort. This suggests, correspondingly to the neural results, that tDCS does not affect performance in the non-emotional letter distractor task.

7.4. Discussion

DLPFC tDCS simultaneously reduced amygdala response and increased frontoparietal activation to unattended fearful faces under conditions of low cognitive effort. These neural effects were accompanied by reduced influence of fearful face distractors on accuracy levels (i.e. a reduced threat response). These results provide the first experimental evidence for a causal inhibitory role of frontoparietal cortical regions on amygdala response to fear/ threat in humans. They also suggest a mechanism of action for treatment effects of tDCS previously observed in clinical trials in emotional disorders.

Animal studies suggest that amygdala reaction to threat is a learned conditioned response which can be extinguished by top-down inhibition from the pre-frontal cortex. In humans, brain imaging studies have revealed hypo-activation of the DLPFC in trait/ state anxiety (85, 123, 124), which is thought to reflect deficient attentional control, particularly in the case of fearful distractors in anxiety disorders (125). The present study demonstrates that enhancing excitability in the DLPFC can abolish amygdala response to threat, whilst simultaneously increasing activity in frontoparietal regions, providing the first experimental evidence for a direct causal relationship between these regions in humans.

The frontoparietal sites revealed by the whole brain analysis include the right angular gyrus, intraparietal sulcus (IPS) and the frontal eye fields (FEF). These frontoparietal regions constitute a well-established attentional control source network (for a review, see (259)), which is thought to facilitate the detection of behaviourally relevant stimuli(260) using spatial(261) or feature(262) based attention modulating control signals. These findings highlight the importance of fronto-parietal-limbic circuitry in the effects of DLPFC tDCS which may be relevant for the behavioural effects reported previously(250) and here.

The results from Study One indicate that frontal tDCS has the potential to reduce vigilance to threat in a paradigm validated to predict clinical response to anxiolytic treatment (250). The underlying neural mechanism for this is proposed to be increased suppression of amygdala threat response through improved regulation from a top-down attentional control network. Depressed and anxious patients have increased amygdala

activation to emotional stimuli (66, 118) and decreased prefrontal activation in response to cognitive tasks (119). There is a dynamic relationship between cortical regions implicated in attentional control and the amygdala which varies as a function of task demand. Bishop et al. combined these two cognitive aspects in an fMRI investigation of an attentional control task with fearful distractor stimuli; the results indicating that trait anxiety is inversely correlated with increased frontal activation and decreased amygdala activation (85). Crucially, these results are only apparent under conditions of low cognitive effort. In other words, when a task does not fully occupy attentional resources there is spare processing capacity available and therefore increased distractibility, particularly by salient (emotional) stimuli (127). In terms of the frontal-amygdala circuit the attentional control network comes 'online' to exert top-down control over amygdala responses to salient non-task stimuli. This top-down control function is impaired in trait anxiety (reflected in hypoactive PFC) contributing further towards a hyperactive amygdala. While these findings shed light on the prefrontal-amygdala process of emotional regulation they are, as with most neuroimaging investigations, correlational. Our findings advance our understanding of this process using tDCS as a causal neuromodulatory probe.

Bishop et al. (85) found that high anxious subjects showed increased amygdala and decreased frontal activation to fearful distractor faces under conditions of low cognitive effort, compared to low anxious subjects. Our anxious sample showed a similar profile under the sham stimulation condition. As hypothesised, we found that tDCS reversed the direction of activations so that after stimulation our high anxious group more closely resembles the low anxious subjects in Bishop et al., with reduced amygdala and increased frontoparietal response. A similar effect was seen in behavioural responses; trait anxiety was associated with increased errors during fearful face distraction in the previous study

(Bishop et al.), presumably because active threat processing distracted behavioural performance. Here we found that tDCS reduced the influence of fearful face distraction on error rate; as after stimulation our high anxious participants more closely resemble the behavioural pattern seen in low anxious participants. There were no effects of tDCS on neural activity and behaviour in the non-emotional version of the task, suggesting that the effect may be specific to the top-down control of responses to threat, rather than a general improvement in attentional control.

A number of recent studies have indicated that repeated administration of prefrontal tDCS may be an effective treatment for depression (154-156). One recent individual patient meta-analysis (Brunoni, personal communication ahead of publication) indicated that two weeks of daily treatment with tDCS had similar efficacy to antidepressant drugs and in one direct comparison of drug treatment with tDCS, tDCS appeared to have faster efficacy (156). The recruitment of frontoparietal based control over amygdala activity in response to threat seen here provides a potential mechanism underpinning these therapeutic actions. Previous studies have also reported an alteration of prefrontal-amygdala circuit activity with invasive deep brain stimulation (DBS) (263), repetitive transcranial magnetic stimulation (rTMS) (135, 136) and SSRIs in treatment responders (78), suggesting this may be a cross-modality marker of response for treatment of emotional disorders. The effect of antidepressants on neural and behavioural response to threat is typically seen prior to changes in clinical state and has been suggested to influence reactions to on-going environmental stimuli and stressors and affect subjective state over time. Our study is the first to non-invasively increase frontoparietal and reduce amygdala activity in response to emotional information, which is applicable to the frontal-amygdala circuit of emotional processing.

The current results in anxious subjects revealed an effect of DLPFC tDCS on a neural biomarker relevant to clinical depression and anxiety disorders. Taken together with the findings from Study One, this indicates a potential neurocognitive mechanism (reduced fear vigilance facilitated by increased frontoparietal and reduced amygdala activation) that may partially mediate the reported findings of clinical efficacy of prefrontal tDCS in the literature. This neural biomarker may have potential to test and benchmark novel stimulation protocols at the development phase, in order to optimize treatment efficacy for depression and anxiety disorders. Furthermore, this study provides the first evidence that directly increasing excitability of the DLPFC leads to a reduced response to threat in the amygdala, confirming evidence from pre-clinical animal studies and correlational studies with neuroimaging in humans. This is the first time the prefrontal-amygdala network has been probed non-invasively using electrical brain stimulation. Imaging research is limited in general by its correlational nature. Hence, the value of this study is that it offers causal evidence of the relationship between the DLPFC and the amygdala in threat processing.

Chapter Eight

General discussion

8.1. Summary of findings

This thesis aimed to explore the mechanisms of action underlying the use of frontal cortex tDCS in the treatment of mood and anxiety disorders. More specifically, the objective was to investigate whether tDCS affects emotional processing acutely in a similar way as previously demonstrated with other antidepressant and anxiolytic treatments. In order to do this a series of studies were conducted looking at the effect of tDCS on emotional processing and its underlying neurocircuitry.

In the first study, the effects of a single session of frontal cortex tDCS on a number of measures of emotional processing were investigated. It was found that bipolar balanced tDCS significantly reduced vigilance towards fearful faces compared to sham stimulation. These effects were found in healthy volunteers, in the absence of any changes in subjective mood or state anxiety, suggesting that the modulation of attentional vigilance away from threatening stimuli may be a direct effect of tDCS. Such effects may be involved in the clinical efficacy of tDCS in clinical trials for depression. There were no effects of tDCS on the other emotional processing tasks, suggesting that the effect of tDCS may be specific to fear processing and have an anxiolytic rather than antidepressant effect. This study employed two active tDCS electrode montages and results favoured a bilateral balanced montage, thereby refining parameters for future studies.

Following on from the reduction in threat vigilance found in the first study, a second investigation was conducted to examine whether tDCS could augment the effects of ABM training. To assess this a single session of tDCS was delivered concurrently with a computerised ABM training task. Measurements of attentional bias and a lab based stressor revealed that the basic bias modification training effect was not achieved and in addition, there were no differences in these measures between the active and sham tDCS groups. This study also included measurements of HPA axis function via the recording of CAR pre and post stimulation/ ABM and found that a single session of tDCS reduced CAR the day after stimulation compared to sham stimulation (regardless of ABM condition). This study was followed up with a research trip to the University of Western Australia, where an additional tDCS/ABM study was carried out in a trait anxious group. Again, bias modification was not achieved and the difference between the tDCS groups was also not significant. At this point it was decided that tDCS may be better suited for 'at rest' administration.

The final investigation combined neuroimaging and tDCS to elucidate the neural underpinnings of the behavioural reduction in threat vigilance found in the first study. A single session of tDCS had the hypothesised effect on neural response to threat in trait anxious females, with increased frontoparietal activation and a corresponding reduction in amygdala activation to unattended fearful faces. This was accompanied with an improvement in letter search accuracy in the fearful trial types.

Taken together these findings demonstrate, within two different paradigms, that a single session of frontal cortex tDCS reduces vigilance to threatening information. The behavioural results suggest, and neuroimaging results support, the model of improved top-

down control of limbic responses to threat, which may be an important component of the mechanism of action of the effects of tDCS observed in clinical trials.

8.2. TDCS and emotional processing

8.2.1. RDoc classification of findings

In order to reorient the perspective of this research and consider the wider implications of the findings, it is useful to apply the RDoc classification to the positive findings from this thesis. The behavioural reduction of vigilance to threat observed in Study One fits into the Negative Valence Systems domain, in the Acute Threat ("Fear") construct and is summarised at the *behaviour* level with the elements *facial expressions* and *response time,* also at the *circuits* level with the element *amygdala* and also at the *molecules* level with the elements *GABA* and *Glutamate* (for the effects of tDCS). These results also fit into the Cognitive Systems domain, in the Visual Perception construct and are summarised at the *paradigms* level with the elements *emotion expression identification* and *face identification*. The reduction in CAR observed in Study Two fits into the Negative Valence Systems domain, Potential Threat ("Anxiety") construct and is summarised at the *molecules* level with the element *cortisol* and also summarised at the *physiology* level, with the element *average cortisol levels* (perhaps the RDoc matrix would benefit from the addition of CAR in addition to average cortisol levels). The CAR reduction also fits into the Arousal and Regulatory Systems domain and is summarised at the *circuits* level with the element *HPA axis* and also summarised at the *molecules* level with the element *cortisol*. Finally the imaging results from Study Four fit into the Cognitive Systems domain, Cognitive Control construct and is summarised at the *paradigms* level with the element *visual search,* at the *behaviour* level with the element *distractibility,* at the *circuits*

level with the element *DLPFC* and at the *molecules* level with the elements *GABA* and *Glutamate*. They also fit into the Cognitive Systems domain, Working Memory construct, and Limited Capacity sub-construct and summarised at the *circuits* level with the element *DLPFC* and at the *molecules* level with the elements *GABA* and *Glutamate*. Both the behavioural and imaging results fit into the Social Processes Domain, Social Communication construct, Reception of Facial Communication sub-construct and is summarised at the *paradigms* level with the element *other attention paradigms*, at the *behaviour* level with the element *identification of emotion* and at the *molecules* level with the element *GABA.*

Therefore to summarise, the domains and constructs in which the findings from this thesis may be applicable across a range of emotional disorders are:

- Negative Valence Systems domain
    - Acute Threat ("Fear") construct
    - Potential Threat ("Anxiety") construct
- Cognitive Systems domain
    - Visual Perception construct
    - Cognitive Control construct
    - Working Memory - Limited Capacity construct
- Arousal and Regulatory Systems domain
    - Circadian Rhythms construct
- Social Processes domain
    - Social Communication construct, Reception of Facial Communication sub-construct

8.2.2. Specific effect on threat processing - anxiolytic effect?

The initial healthy volunteers investigation (Study One) examined the acute effects of frontal tDCS on a battery of behavioural tests previously validated for antidepressant and anxiolytic treatment effects. This cognitive battery included tests of facial expression recognition, emotional memory and vigilance to threat. In order to investigate potential mechanisms of action of tDCS in the treatment of mood and anxiety disorders, the cognitive effects of tDCS must be compared to those seen with other antidepressant/anxiolytic treatments. Study One found specific effects of frontal tDCS on a dot probe paradigm which measures attentional vigilance to threat. This is a phenomenon which has well established relevance to attentional biases in anxiety disorders (126); and is thought to emerge from deficient attentional control in trait anxiety (101). The reduction in threat vigilance observed was similar to that seen with acute administration of the benzodiazepine diazepam in the same cognitive paradigm (114) and also from acute treatment with an exposure based CBT intervention for panic disorder (115), the latter effect proving to be predictive of later treatment response. Since a similar pattern of effects on this task has been observed previously both in studies examining the effects of anxiolytic drugs on healthy volunteers and patients undergoing anxiolytic treatment, it is possible that the effects shown in the present study reveal a common mechanism of action for previously reported effects of tDCS on standard measures of mood in clinical trials (156).

There were no effects of a single session of frontal tDCS on the other measures of emotional processing in the battery under investigation in Study One, which had previously been modified by the administration of antidepressant drugs (264). Whereas

the other emotional processing tasks (such as facial expression recognition, emotional categorisation and emotional memory) are typically used to assess cognitive effects of antidepressant treatment (173, 196), the dot probe task is mainly used to measure anxiolytic treatment (114, 197). Additionally, antidepressant drugs have also been shown to modify vigilance to threat (193), which is consistent with their use in the treatment of anxiety disorders. This suggests that the mechanism of action of tDCS in clinical trials may affect a different or narrower cognitive domain than antidepressant drugs, one which is more specific to fear processing and trait anxiety. The cognitive neuropsychological framework suggests that antidepressant drugs affect the stimulus driven (bottom-up) aspects of emotional processing and psychological interventions affect the top-down attentional control aspects of emotional processing (265), which is supported with neuroimaging evidence (122). This cognitive dichotomy in treatment effects lends support for the indication that frontal tDCS may affect specifically anxious symptoms, rather than both anxious and depressive symptoms. This could be tested in clinical trials of depressed patients by taking state and trait anxiety measures at baseline and follow up. In addition, separating the groups between anxious and non-anxious depression could test for a differential effect of tDCS. Anxious depression is more difficult to treat than non-anxious depression (266) and so if tDCS specifically treated this facet of depressive symptoms there is much to be gained clinically.

8.2.3. Effects of tDCS on neural circuits of emotion

The behavioural findings from Study One had a theoretical basis in the modification of the cortico-limbic circuit of emotional processing, which has strong evidence from animal models (72, 73, 76) and neuroimaging (79-82). The PFC is an area implicated in dysfunctional network integration in depressed patients, in terms of disrupted top-down

control of the amygdala by the PFC, shown in fMRI studies on abnormal responses to negative feedback (171) and emotional/ non-emotional information processing (119). Depressed and anxious patients have increased amygdala activation to emotional stimuli (66, 118) and decreased prefrontal activation in response to cognitive tasks (119). This aberrant pattern of activation is also a feature of high trait anxious healthy individuals when processing threat related stimuli (123). It was proposed that the behavioural reduction in threat vigilance in Study One was facilitated by an increase in frontal top-down activation and a corresponding reduction in limbic bottom-up, fear stimulus driven activity.

The subsequent imaging task selected for Study Four was chosen on the basis of its relevance to trait anxiety and attentional control. Furthermore, a trait anxious group was recruited in an attempt to replicate trait anxious findings in the sham tDCS condition and show that these phenomena can be modified by frontal tDCS. Bishop et al.'s (85) high trait anxious group show higher amygdala activation to fearful faces and lower DLPFC activation, when compared to a low trait anxious group, under conditions of low cognitive effort. The high anxious amygdala effect under low cognitive effort was replicated in this thesis in the sham tDCS condition and there was a subsequent modification of this activity pattern resulting from frontal tDCS, increasing frontoparietal activation and reducing amygdala activation to unattended fearful faces, compared with sham stimulation.

This neural modification can be compared to fear extinction in animal models, which is mediated by the prefrontal cortex (73). In the instance of fearful faces, the lifelong conditioned response in humans emerging from interactions in society, is one of threat, which is signalled by amygdala activation. Over time, repeated experimental

presentation of fearful faces with no adverse impact results in a reduction in fear responses, just like extinction learning. This 'extinction learning' is proposed to be augmented by frontal tDCS and is supported by the imaging results. Previous studies have also reported an alteration of prefrontal-amygdala circuit activity with invasive deep brain stimulation (DBS) (88, 89), repetitive transcranial magnetic stimulation (rTMS) (135, 136) and SSRIs in treatment responders (86), suggesting this may be a cross-modality marker of response for treatment of emotional disorders.

This thesis provides the first evidence that directly increasing excitability of the DLPFC leads to a reduced response to threat in the amygdala, confirming evidence from pre-clinical animal studies and correlational studies with neuroimaging in humans. This is the first time the prefrontal-amygdala network has been probed non-invasively using electrical brain stimulation. Imaging research is limited in general by its correlational nature. Hence, the value of this data is that it offers causal evidence of the relationship between the DLPFC and the amygdala in threat processing. This presents strong evidence for the mechanism of action of tDCS in reducing behavioural threat vigilance (Study One) and also a proposed neural correlate for the effects of tDCS observed in clinical trials.

8.2.4. Clinical relevance of findings

From the considerations of cost, ease of use and favourable side effect profile, tDCS is an attractive novel treatment for depression. The development of home use devices broadens the potential clinical capacity of this intervention. However, unlike rTMS, tDCS does not yet have FDA approval for the treatment of depression and its use is still experimental. In order to ascertain whether this is a treatment worthwhile progressing into clinical availability, further knowledge of its mechanism of action is necessary.

A current mission in psychiatric research constitutes the identification of reliable treatment response markers to refine patient groups in research and guide clinical treatment choices. Clinical trials of tDCS have shown mixed results (for a review see (153)), which may be a result of parameter heterogeneity or yet unidentified sub-types of depression which respond differently to tDCS. However, an upcoming individual patient data meta-analysis of 289 tDCS treated patients (Brunoni, personal communication ahead of publication) indicates that a standard course of treatment with tDCS (10 sessions over two weeks) has a similar level of efficacy as antidepressant drugs. The behavioural, neural and adrenal function effects of tDCS presented in this thesis suggest an anxiolytic rather than antidepressant effect, although MDD and anxiety disorders are highly comorbid and have many overlapping symptoms. It is possible that the reduction of depressive symptoms seen in clinical trials of tDCS is actually because of a reduction in symptoms of a comorbid anxiety disorder. This is supported by a study which found that during negative imagery visualization, anodal tDCS leads to decreased cortisol levels and increased vagal activity, measures which reflect stress regulation (199). Clinical trials of tDCS for the treatment of depression should include measures of state/ trait anxiety to test this theory. It would also be interesting to measure the effects of tDCS on anhedonia, which is present in depression but not anxiety disorders as this would provide a useful distinction between the effects of tDCS on the two types of disorder. If tDCS does specifically affect symptoms of anxiety disorders then a comorbid anxiety disorder or high baseline trait anxiety could prove a key biomarker for treatment response from tDCS in depression. Hence, trait anxiety could prove a successful pre-treatment patient selection strategy for further clinical trials of tDCS and eventually perhaps a guide to clinical treatment selection.

The effect of tDCS on CAR is of clinical relevance, because of the association between cortisol levels and stress (233). High cortisol levels are associated with various psychiatric disorders (234) and antidepressant treatment has resulted in reduced CAR in patients (239). A single session of frontal tDCS reduced CAR the day after testing, compared to baseline. Salivatory cortisol sampling is straightforward and can be carried out by patients at home, increasing opportunities for clinical application.

This thesis presents the first neuroimaging evidence of frontal tDCS modulating amygdala response to threat. This has implications for understanding the acute mechanisms of action of tDCS in the treatment of depression and anxiety disorders and suggests that an imaging measurement in clinical trials may be a useful addition in terms of characterising acute predictors of treatment effects. If the acute neural effects of tDCS can be related to subsequent treatment response this would prove a powerful neural biomarker.

Although the combined administration of tDCS and ABM did not show much promise in this data, the proposed similarity of the tDCS mechanism of action to extinction learning opens up new possibilities in terms of adjunct treatments. Psychological interventions which target extinction type processes, such as exposure based therapies, have the potential for augmentation with tDCS. One promising exposure based CBT intervention for panic disorder showed similar reductions in vigilance to threat as tDCS (115) and may prove a useful clinical adjunct.

In summary, the results from this thesis propose the use of bilateral-balanced stimulation montages, inclusion of measures of vigilance to threat, attentional control, state/trait anxiety and HPA axis function in future clinical trials of tDCS for depression. Together, these measures could help elucidate the mechanisms of action and improve how tDCS is clinically implemented.

8.3. Attentional bias modification

The attentional bias modification studies (Study Two and Study Three) in this thesis, on both counts, did not show combined effects of tDCS and ABM. This challenges previous evidence from Clarke et al. (179) that frontal tDCS augments bias modification. Even the opportunity to follow up the initial null finding with a collaboration involving harmonisation of parameters between the design in Study Two and the Clarke et al. study did not improve the ability to detect an effect.

There were some remaining parameter differences between the design in Study Three and Clarke et al. study which could explain the difference in findings. The Clarke et al. study used a different electrode montage to the present study. After the parameter optimisation in Study One, all the subsequent studies in this thesis used a bipolar balanced DLPFC stimulation montage. Clarke et al. used a unipolar montage with an extra-cephalic reference (cathodal) electrode on the neck. Both montages stimulate the left DLPFC with the anodal electrode. However, this study also stimulated the right DLPFC with the cathodal electrode. In addition, the difference in electrical field and current density/ directionality from these two montages could produce different effects on cortical excitability at locations distal from the electrode location. Furthermore, Clarke et al. used a current strength of 1mA in their study, whilst the studies in this thesis used 2mA based

on a review of the depression literature. The variability of effects of tDCS at different current strengths is still under evaluation, with a recent study indicating that 20 mins of 2mA cathodal motor cortex stimulation actually had excitatory effects on motor evoked potentials, whereas 20 mins of 1mA cathodal stimulation had inhibitory effects (201). It is possible that 2mA of bipolar balanced tDCS has different effects to 1mA unipolar tDCS, but it is impossible to confirm this without directly comparing these two stimulation protocols.

Nonetheless, these results are not promising for attentional bias modification as a potential treatment. A single session of ABM has achieved bias modification and transfer effects on lab based stressors in healthy volunteers (210) and high trait anxious groups (267). However, to work as a treatment it may require daily administration over a course of several weeks (48), an effect which is mediated by the prefrontal cortex (211). It is of note that the basic bias modification training effect was not achieved in either of the ABM studies in this thesis, regardless of tDCS condition, so it is understandable that subsequent tests of emotional vigilance and lab based stressors failed to detect an effect. It is argued that although achieving the bias modification is not always possible with ABM, when it is successfully achieved then the positive effects on lab based stressors are reliably attained (227). Therefore, future ABM research should focus on new methods of modifying biases that prove more reliable but based on the findings from this thesis, bipolar balanced tDCS does not facilitate bias modification in this paradigm.

8.4. Methodological considerations

8.4.1. After-effects of tDCS and parameter optimisation

Since the behavioural and imaging tasks were conducted with a delay of ~22 and ~9 minutes after the end of stimulation, it is conceivable that, in some individuals, the physiological after-effect of tDCS may have already decayed by that time. The duration of after-effects of frontal tDCS has not been studied systematically. However, in motor cortex studies, the same stimulation protocol used here (2mA, 20 minutes) had effects lasting for at least 90 minutes after stimulation offset (201). In Study One and Study Four, the tasks were completed before 45 mins had elapsed from the end of stimulation. Hence, testing was conducted reliably within the likely time window of tDCS after-effects.

The review of the clinical tDCS literature revealed massive heterogeneity in the stimulation parameters used across trials. This may explain the heterogeneity of clinical results and underlines how difficult it is to perform meta-analyses of the data. There is also uncertainty surrounding the exact action of tDCS, with a controversial paper showing that, contrary to popular belief, 2mA of cathodal tDCS resulted in excitatory effects (201). This makes parameter choice more difficult, as the effects of current densities may not be linear, and small changes in current could convert an effect from excitatory to inhibitory. 2mA of cathodal tDCS did not appear to increase activation in the right DLPFC in the imaging measures from Study Four. However, cathodal stimulation in this study was part of a bipolar montage (with anodal stimulation of the right DLPFC), rather than a unipolar montage (e.g. with an extra cephalic reference) and therefore does not isolate the effects of cathodal stimulation on the cortex. Computational modelling has been used to estimate the electrical fields of the various electrode montages employed in tDCS (268) but the electrode montages, stimulation duration and current strength used in research are mainly

based on prior convention. In an ideal world, a full programme of parameter optimisation would precede any clinical investigation of tDCS. However, funding and time constraints mean that this has been limited in practice. In fact, this thesis presents one of the first published attempts to refine frontal cortex tDCS parameters by conducting a study using two active electrode montages (269). It was found that bipolar balanced stimulation was superior to placebo in reducing vigilance to threat, whilst bipolar unbalanced was not. However, the two active montages were not statistically different from each other and effects in the bipolar unbalanced condition were halfway between the sham and bipolar balanced conditions, suggesting a partial effect probably emerging from the common left DLPFC anode electrode. This makes comparisons difficult, in a similar manner as dose finding studies in clinical drug development. Nonetheless, the value in phase I healthy volunteer trials is that parameters can be tested and refined relatively more quickly than a full scale clinical trial and the data in this thesis supports a bilateral balanced electrode montage for reductions in vigilance to threat.

8.4.2. Healthy volunteer experimental medicine models

All the studies used a phase I, healthy volunteer experimental medicine model to explore the mechanisms of action of frontal tDCS, although in the last two studies otherwise currently healthy participants were selected on the basis of high trait anxiety. The acute effects of antidepressant treatment on emotional processing are seen in healthy volunteers (270) and patients (166) alike, suggesting a common mechanism. The use of healthy volunteers also allows the basic cognitive effects of tDCS to be investigated without the potential confound of symptom improvement and avoids ethical concerns about randomising patients to receive sham stimulation. In addition, healthy volunteer studies

are much quicker to recruit and execute allowing efficient parameter optimisation, as was seen in Study One.

After parameter optimisation, the selection of volunteers on the basis of trait anxiety allowed some cognitive aspects of trait anxiety and anxiety disorders to be probed and comparisons to be made with other studies which group their volunteers according to trait anxiety. This gives further clinical relevance to the investigation without the need to recruit patients and is a useful next step in defining suitable behavioural and neuroimaging tasks, as per Study Four. However, further studies should include patient groups and involve full treatment interventions, in order to relate the acute findings to subsequent treatment response.

8.4.3. Dot probe reliability

The reliability of the dot probe measure has been called into question in the literature (248, 249), which casts doubt over the findings on vigilance to threat presented in Study One. In fact, the pooled data analysis in Chapter Six revealed that vigilance to fear was much smaller when looking at baseline data from Study Three. There are some potential explanations for this, as the baseline faces dot probe task was carried out after the baseline words dot probe task in Study Three. This could have resulted in habituation to fearful stimuli, which reduces vigilance, similar to extinction effects in conditioning. The findings from Study Three and the pooled analysis did reveal an effect of time on vigilance, with fear vigilance reliably reducing over time, regardless of tDCS/ ABM intervention. This suggests a strong habituation effect to fearful stimuli which is supported by the neuroimaging literature (228). In addition, the participants from Study Three were exclusively first year psychology students while the participants from Study One

constituted a broader demographic. The imaging results add some confidence to the behavioural findings from Study One. Nonetheless, the behavioural effect from Study One should be replicated in a larger sample and alternative measures of vigilance should be employed in future studies.

8.5. Final remarks

The data presented in this thesis propose an anxiolytic-like profile of frontal tDCS, supported by behavioural data, neuroimaging and measures of adrenal function. The current phase I results reveal an anxiolytic-like effect of frontal tDCS on cognitive and neural biomarkers relevant to clinical anxiety disorders, and indicate a potential neurocognitive mechanism (reduced fear vigilance) and an underlying neural mechanism (increased top-down control of amygdala response) that may partially mediate the reported findings of clinical efficacy of prefrontal tDCS in the literature.

The next logical step is to assess whether the effects reported here in healthy volunteer and trait anxious groups can be found in a patient group, and if they can predict response to treatment of depression and/ or anxiety disorders. The results from this thesis support the use of bipolar-balanced DLPFC stimulation paradigms and measurements of attentional bias, attentional control and adrenal function. These measurements have been identified as a marker of efficacy in other treatments and so this protocol could have a similar effect in a clinical population, where reductions in negative biases have been shown to predict later clinical improvement (115, 166). The focus of this work was to investigate acute effects of tDCS, by testing for an induced change in emotional processing and neural activity similar to what has previously been observed acutely with antidepressant or anxiolytic treatment, or correlated with lower trait anxiety. However, it

will also be necessary to evaluate whether these effects, seen acutely, can be extended over time with repeated interventions, as occurs with antidepressant or anxiolytic treatment. Results from this thesis do not support the combination of tDCS and ABM training, however, it could be useful to examine the efficacy of frontal cortex tDCS as an adjunct to other interventions which target attentional biases by using extinction techniques (e.g. exposure therapy) or attentional control improvements (e.g. cognitive control training (229)).

In summary, this thesis offers an experimental medicine framework that could be used for future testing, refining and validating the novel intervention of prefrontal tDCS to mitigate mood and anxiety disorders. In contrast with the more empirical approach to devising tDCS interventions that characterizes the existing literature (as reflected in the great heterogeneity of stimulation parameters), this thesis outlined a principled approach, from parameter optimisation to behavioural results defining the cognitive aspects of imaging measures. From this an anxiolytic-like stimulation profile is described, using a laboratory test of proven clinical predictive utility, supported by a priori hypothesised neural correlates. Hence, the value of this work is that it offers a principled path towards testing and benchmarking novel stimulation protocols at the development phase, either in healthy volunteers or in clinical populations, in order to optimise treatment efficacy for mood and anxiety disorders.

Appendices

Appendix I: Literature review, parameters of successful studies used in design

Citation	N	Design	Amplitude	Duration	Sessions
Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., & Fregni, F. (2008). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. The International Journal of Neuropsychopharmacology, 11(02), 249-254. doi: doi:10.1017/S1461145707007833	40	RDB	2	20	10
Brunoni, A. R., Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Boggio, P. S., . . . Priori, A. (2011). Transcranial direct current stimulation (tDCS) in unipolar vs. bipolar depressive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35(1), 96-101. doi: 10.1016/j.pnpbp.2010.09.010	31	OL	2	20	10
Brunoni, A. R., Valiengo, L., Baccaro, A., Zanao, T. A., de Oliveira, J. F., Vieira, G. P., . . . Fregni, F. (2011). Sertraline vs. ELectrical Current Therapy for Treating Depression Clinical Trial - SELECT TDCS: Design, rationale and objectives. Contemporary Clinical Trials, 32(1), 90-98. doi: 10.1016/j.cct.2010.09.007	120	RDB	2	30	10
Dell'Osso, B., Zanoni, S., Ferrucci, R., Vergari, M., Castellano, F., D'Urso, N., . . . Altamura, A. C. (2012). Transcranial direct current stimulation for the outpatient treatment of poor-responder depressed patients. European Psychiatry, 27(7), 513-517. doi: 10.1016/j.eurpsy.2011.02.008	23	OL	2	20	10
Ferrucci, R., Bortolomasi, M., Brunoni, A., Vergari, M., Tadini, L., Giacopuzzi, M., & Priori, A. (2009). Comparative benefits of transcranial direct current stimulation (TDCS) treatment in patients with mild/moderate vs. severe depression. Clinical Neuropsychiatry, 6(6), 246-251.	13	OL	2	20	10
Ferrucci, R., Bortolomasi, M., Brunoni, A., Vergari, M., Tadini, L., Giacopuzzi, M., & Priori, A. (2009). Comparative benefits of transcranial direct current stimulation (TDCS) treatment in patients with mild/moderate vs. severe depression. Clinical Neuropsychiatry, 6(6), 246-251.	19	OL	2	20	10
Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Salvoro, B., Giacopuzzi, M., . . . Priori, A. (2009). Transcranial direct current stimulation in severe, drug-resistant major depression. Journal of Affective Disorders, 118(1–3), 215-219. doi: 10.1016/j.jad.2009.02.015	14	OL	2	20	10
Fregni, F., Boggio, P. S., Nitsche, M. A., Rigonatti, S. P., & Pascual-Leone, A. (2006). Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression. Depression and Anxiety, 23(8), 482-484. doi: 10.1002/da.20201	18	RDB	1	20	5
Fregni, F., Boggio, P.S., Nitsche, M.A., Marcolin, M.A., Rigonatti, S.P., Pascual-Leone, A., 2006. Treatment of major depression with transcranial direct current stimulation. Bipolar Disord. 8, 203–204.	10	RDB	1	20	5
Loo, C. K., Alonzo, A., Martin, D., Mitchell, P. B., Galvez, V., & Sachdev, P. (2012). Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. The British Journal of Psychiatry, 200(1), 52-59. doi: 10.1192/bjp.bp.111.097634	64	RDB	2	20	15
Rigonatti, S. P., Boggio, P. S., Myczkowski, M. L., Otta, E., Fiquer, J. T., Ribeiro, R. B., . . . Fregni, F. (2008). Transcranial direct stimulation and fluoxetine for the treatment of depression. European Psychiatry, 23(1), 74-76. doi: 10.1016/j.eurpsy.2007.09.006	42	RDB	2	20	10

Appendix II: Description of materials

Researcher administered tasks and questionnaires

SCID screening: Participants are screened for current or previous psychiatric disease and substance abuse / dependence using the Structured Clinical Interview for DSM-IV Disorders (SCID;(271))

National Adult Reading Test (NART;(272)): Participants' verbal IQ / education level is estimated using the NART.

Self-report questionnaires

Trait measures:

- Eysenck Personality Questionnaires (EPQ;(273))

State measures:

- Beck Depression Inventory (BDI; (10))
- Positive and Negative Affect Schedules (PANAS; (182))
- Visual Analogue Scales (VAS; (274))
- State-Trait Anxiety Inventory (STAI; (24))

Appendix III: Study methods

Transcranial direct current stimulation

TDCS is a non-invasive method of delivering brain stimulation using weak electrical current, generated by a battery powered stimulation device (147) and passed through rubber electrodes which are fixed to the scalp with a rubber band. The method has been used in hundreds of studies to date worldwide and is considered safe, with minimal negative side-effects reported. A recent meta-analysis confirmed this as a very low risk method (153). Tested safety thresholds (from studies in rats) are two orders of magnitude above what is possible with the stimulation devices used (275). In addition, for all studies, protocol for stimulation will stay within well-established parameters detailed in peer reviewed publications by leading experts in the field of tDCS (276).

After screening for potential contraindications to tDCS using a standardised safety questionnaire, participants will be usually randomly assigned to the active group or the control group. The control group will receive sham stimulation. For the active stimulation 2mA of electrical current will be applied to the scalp via rubber electrodes encased in saline soaked sponges (25cm²). Electrodes will be placed in the chosen location using the 10-20 system of electroencephalogram (EEG) electrode location and an EEG cap. The stimulation will last for 20 minutes, whilst the participant is at rest or completing a computerised task/training. For sham stimulation using a sham setting on the device, the current is applied intermittently and at lower amplitude, to create the same itching/tingling sensation that is usually perceived with tDCS. During sham stimulation only a small current pulse occurs every 550 ms (110 μA over 15 ms) instead of the stimulation current with the peak current lasting for 3 ms. This current pulse enabled an impedance control which reliably detects bad electrode contact or electrode disconnection. In sham

stimulation average current over time is not more than 2 μA, which has no therapeutic effect (147). The idea is to raise the resting potential of the neurons in the left DLPFC, making brain activity in this area more likely for up to an hour after stimulation. In addition, the long term efficacy of certain receptors (NMDA receptors) in the stimulated region may be increased, though this is usually only observed with repeated stimulation.

Participants are given information about what to expect from the stimulation, in terms of sensory perception and any (extremely rare) side effects.

Salivatory Cortisol

In Study Two HPA axis activity was assessed on the morning of testing (before tDCS/ABM) (day 1), the morning after testing (day 2) and at four week follow up (day 28), using the salivary cortisol awakening response (236). For each CAR measurement, participants were instructed to provide three saliva samples (using salivettes, Sarstedt Ltd., Nümbrecht, Germany) taken in their own home immediately upon waking and subsequently every 15 min until 45 min post-waking. Saliva samples were delivered in person or sent by first class post and once received, stored at 4 °C prior to analysis. Cortisol was measured using a commercial ELISA (Salimetrics Europe Ltd., Newmarket, UK), within 7 days of sample collection after which samples were destroyed.

Cortisol Instructions given to participants:

MORNING [28] DAYS AFTER STUDY

EARLY MORNING SALIVA SAMPLES – INSTRUCTIONS

We need 3 samples of your saliva first thing in the morning.

We collect the saliva samples in plastic tubes which have a synthetic swab in them. Please open the tube take the swab out of the tube and put it in your mouth for 1 minute. You do not need to chew the swab, but rolling it about in your mouth may help to stimulate saliva production. Put the swab back in the tube and wrap the three tubes in the small plastic bag provided.

PLEASE DON'T:

1. Please do not have any alcohol the night before.

2. You should not have anything to eat or drink for the duration of the test which lasts half an hour from the time you wake up.

3. You should not indulge in strenuous activity on the morning of the test.

4. Please do not clean your teeth until after you have collected all 3 samples.

Otherwise follow your usual morning routine.

PLEASE DO:

1. You should obtain your first saliva sample immediately after waking while lying in bed (use tube 0).

2. You should obtain the next sample 15 minutes after waking up (use tube 15).

3. You should provide the final sample at 30 minutes after waking up (use tube 30).

4. Please answer the online questionnaires (http://tinyurl.com/tdcsmorning28daysafterstudy) which will help us to interpret the results.

PLEASE RETURN THE THREE SAMPLE TUBES ON THE DAY ON WHICH YOU COLLECT THE SAMPLES IN THE ADDRESSED AND STAMPED ENVELOPE PROVIDED.

Functional Magnetic Resonance Imaging

Blood oxygenation level dependent (BOLD) contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens Magnetom TrioTim syngo

MRB17 with a head coil gradient set. Each image was made up of 45 interleaved 3mm thick slices, interslice gap 1mm; field of view 25x25cm; matrix size 64 x 64; flip angle 87° echo time (TE) 30ms; voxel bandwidth 2368 Hz/Px; acquisition time (TA) 2.3 s; repetition time (TR), 2710ms. Slice acquisition was interleaved and covering the whole brain with an additional z shim to reduce distortion in the orbitofrontal cortex. Data were acquired in 6 scanning runs of 5 min. The first 5 volumes of each run were discarded to allow for T1 equilibration effects.

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB Software Library, www.fmrib.ax.ac.uk/fsl). Registration to high resolution structural and standard space was carried out using FLIRT (255, 256). Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear registration (257, 258).

Attentional bias modification training/ Dot probe words list

Appendix

Stimulus Pairs

Subset A	Subset B
Suffer–Parked	Grave–Filed
Wound–Dried	Cancer–Saddle
Attacks–Physics	Desperate–Variables
Victims–Smelled	Danger–League
Tease–Aisle	Defeat–Museum
Discouraged–Connections	Shot–Cars
Gloomy–Pastel	Trauma–Enjoin
Tormented–Mythology	Kill–Shop
Panicky–Clarets	Worried–Context
Insecure–Fetching	Powerless–Multitude
Horror–Wagons	Devastated–Stagecoach
Dead–Data	Angry–Curve
Afraid–Detail	Threat–Varied
Bitter–Handle	Severe–Recall
Evil–Hill	Sinister–Integral
Fright–Sipped	Assault–Bottles
Disease–Remarks	Lost–Read
Worthless–Batteries	Despised–Tomatoes
Rejected–Quantity	Humiliated–Waterproof
Bomb–Crew	Injury–Holder
Worst–Owned	Intimidated–Coefficient
Catastrophe–Approximate	Awful–Tract
Lethal–Racket	Mourn–Scans
Ignored–Lighted	Scared–Planet
Tragic–Rector	Conflict–Detailed
Terror–Pupils	Dull–Flew
Trap–Tent	Murder–Junior
Hazard–Ballot	Agitation–Fireplace
Hopeless–Feathers	Incurable–Reclaimed
Inadequate–Transition	Stress–Cities
Forlorn–Keyhole	Hostile–Rolling
Coffin–Edited	Pain–Laws
Strangled–Signature	Grieving–Hallmark
Apprehension–Instrumental	Sickly–Tokens
Fear–Note	Cry–Via
Trouble–Evening	Anxiety–Journal
Worry–Inner	Dying–Lists
Enemy–Check	Hurt–Core
Distress–Creature	Mutilated–Decanting
Nausea–Confer	Suffocating–Constituent
Tragedy–Request	Lonely–Jersey
Sad–Pat	Pathetic–Cleaners
Suffered–Recorded	Violent–Thereby
Destroyed–Furniture	Hatred–Fitted
Damage–Campus	Midway–Dismal
Harm–Pond	Futile–Attire
Inferior–Shearing	Deathbed–Softener
Sluggish–Textured	Unhappy–Bridges

Note. The negative word is the first member of each pair.

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