UNIVERSITY OF SOUTHAMPTON

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Medicine

Novel treatment approaches for anxiety disorders: Mindfulness-based approaches

and Transcranial Direct Current Stimulation

by

Daniel Meron

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<u>ABSTRACT</u>

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Anxiety disorders are an important cause of morbidity worldwide. Existing treatments for anxiety disorders have considerable shortcomings and new treatments are needed. Anxiety impairs attentional control through effects on central executive functions, whereas Mindfulness training has effects on executive function and attention. This thesis explores the potential for using mindfulness and transcranial direct current stimulation (tDCS) as treatment modalities for anxiety disorders, beginning with a literature review, and going on to describe a series of investigations in healthy volunteers.The first study compared the effects of two types of mindfulness training: focused attention (FA) *vs.* open monitoring (OM), on attention network function, using the Attention Network Test (ANT). The second study explored the effects of a strengthened, integrated FA and OM mindfulness training on attention to threat, using an antisaccade task. A third study examined the effects of a single session of guided FA *vs.* OM mindfulness on attention to threat (measured using an antisaccade task), during inhalation of air enriched with 7.5% carbon dioxide (CO_2). The fourth study evaluated the effect of a single session of tDCS on attention network function (measured using the ANT). The final study examined the effect of a single session of tDCS on attention to threat (measured using the antisaccade task), during inhalation of 7.5% CO_2. The main findings of these studies are as follows: A literature review demonstrated that Mindfulness-based interventions have a substantial evidence base for efficacy in depression and a growing evidence base in anxiety disorders.   A meta-analysis of randomised controlled trials (RCTs) comparing active *vs.* sham tDCS in depression found that in patients with major depressive episodes, tDCS offers an effective and tolerable alternative to antidepressant medication for those who do not wish to take or cannot take tolerate medication, or cannot tolerate it: current evidence does not support the use of tDCS in treatment resistant depression, or as an augmentation treatment with antidepressant medication or Cognitive Control Training (CCT). There are no published RCTs of tDCS in anxiety disorders. Mindfulness interventions were associated with enhanced executive control function on the ANT, and attenuated the effects of 7.5% CO_2 inhalation on anxiety A single session of tDCS was associated with enhanced executive control function on the ANT, but did not protect against anxiety during inhalation of 7.5% CO_2. These findings suggest tDCS may be best utilised during the early stages of depression treatment pathways, and have implications for future design of mindfulness interventions for anxiety.

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Declaration of Authorship

I, Daniel Meron, declare that the thesis entitled "Novel treatment approaches for anxiety disorders: Mindfulness-based approaches and Transcranial Direct Current Stimulation"
and the work presented in it are my own and has been generated by me as the result of my own original research. I confirm that:

• This work was done wholly or mainly while in candidature for a research degree at this University;

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• Where I have consulted the published work of others, this is always clearly attributed;

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Abbreviations

5-HT	- 5-hydroxytryptamine
ABM	- Acceptance-Based Model of GAD
ABMT	- Attentional Bias Modification Training
ACC	- anterior cingulate cortex
ACT	- Attentional Control Theory
ADM	- Anti-Depressant Medication
AMW	- Avoidance Model of Worry of GAD
ANOVA	- Analysis Of Variance
ANT	- Attentional Network Test
APA	- American Psychiatric Association
ASI	- Anxiety Sensitivity Index
BDI	- Beck Depression Inventory
BMI	- Body Mass Index
BOLD	- Blood-oxygen-level dependent
BPM	- Beats Per Minute
CBT	- Cognitive Behavioural Therapy
CCT	- Cognitive Control Training
CGIS	- Clinical Global Impression of Severity
CO_2	- Carbon Dioxide
CRF1	- Corticotrophin Releasing Factor 1
CT	- Cognitive Therapy
DBP	- Diastolic Blood Pressure
DBT	- Dialectical Behavioural Therapy
DLPFC	- Dorso-Lateral Pre-Frontal Cortex
DMPFC	- Dorso-Medial Pre-Frontal cortex
DSM	- Diagnostic and Statistical Manual of Mental Disorders
DSM	- Diagnostic & Statistical Manual of Mental Disorders
ECT	- Electro-Convulsive Therapy
EDM	- Emotion Dysregulation Model of GAD
EEG	- Electroencephalography
EOG	- Electrooculography
ER	- Error Rate
ERP	- Event-related potential
ESP	- Evidence-Based Synthesis Program
FA	- Focussed Attention
FEF	- Frontal Eye Fields
FFMQ	- The five facet mindfulness questionnaire
FFT	- Fast Fourier transform
FMI	- Frieberg Mindfulness Inventory
fMRI	- Functional magnetic resonance imaging
GAD_7	- Generalised Anxiety Disorder - 7
GAD	- Generalised Anxiety Disorder
GAF	- Global Assessment of Functioning Scale
HA	- High Anxiety
HARS	- Hamilton Anxiety Rating Scale
HDRS	- Hamilton Rating Scale for Depression
HPA	- Hypothalamic-pituitary-adrenal axis
HR	- Heart Rate

Hz	- Hertz
IAPS	- International Affective Picture System
ICD	- International Statistical Classification of Diseases
IPS	- Intra-Parietal Sulcus
IPT	- Inter Personal Therapy
ISI	- Inter-Stimulus Interval
ITI	- Inter-Trial Interval
IUM	- Intolerance of Uncertainty Model of GAD
KIMS	- Kentucky inventory of mindfulness skills
LA	- Low Anxiety
LPFC	- Lateral prefrontal cortex
LPP	- Late positive potential
LTD	- Long Term Depression
LTP	- Long Term Potentiation
M1	- Primary Motor Cortex
MAAS	- Mindful Attention Awareness Scale
mA	- milliampere
MAP	- Mean Arterial Pressure
MBCT	- Mindfulness Based Cognitive therapy
MBI	- Mindfulness-based intervention
MBSR	- Mindfulness Based Stress Reduction
MCI	- Mild cognitive impairment
MDD	- Major Depressive Disorder
MDE	- Major Depressive Episode
MDRS	- Montgomery-Asberg Depression Rating Scale
MEP	- Motor evoked potential
MINI	- Mini-International Neuropsychiatric Interview
mmHg	- millimetres of Mercury
mPFC	- Medial prefrontal cortex
msec	- Millisecond
MTM	- Meta-Cognitive Model of GAD
N_2	- Nitrogen
NAT	- Negative Automatic Thoughts
NICE	- National Institute for Health and Care Excellence
NMDA	- N-Methyl-D-aspartic acid
O_2	- Oxygen
OCD	- Obsessive-Compulsive Disorder
OFC	- Orbito-Frontal Cortex
OM	- Open Monitoring
PANAS	- Positive and Negative Affect Schedule
PASAT	- Paced Serial Addition Task
PD	- Panic Disorder
PET	- Positron emission tomography
PFC	- Prefrontal cortex
PGII	- Patient Global Impression Scale of Improvement
PIM	- Person-identity-matching (task)
POMS	- Profile of Mood States
PPC	- Posterior Parietal Cortex
Pre-SMA	- Pre-supplementary motor area
PSWQ	- Penn State Worry Questionnaire
PTSD	- Post-Traumatic Stress Disorder

RAM   - Resource Allocation Mechanism
rCBF   - regional Cerebral Blood Flow
RC   - Relaxation Control
RCT   - Randomised Controlled Trial
rIFG   - right Inferior Frontal Gyrus
RP   - Relapse Prevention
rTMS   - Repetitive Transcranial Magnetic Stimulation
RT   - Reaction time
SAD   - Seasonal affective disorder
SBP   - Systolic Blood Pressure
SD   - Standard Deviation
SMD   - Standardised mean difference
SSAI   - Spielberger State Anxiety Inventory
SSRI   - Selective Serotonin Re-uptake Inhibitor
STAI   - State-Trait Anxiety Inventory
STAI-T   - Spielberger Trait-Anxiety Inventory
TAU   - Treatment As Usual
TCA   - Tricyclic Antidepressant
tDCS   - Transcranial Direct Current Stimulation
TMS   - Transcranial Magnetic Stimulation
VAS   - Visual Analog Scale
VLPFC   - Ventrolateral pre-frontal cortex
VMPFC   - Ventromedial prefrontal cortex
Vs.   - versus
WAT   - Wells Attentional Training
WHO   - World Health Organisation}

1. CHAPTER 1: INTRODUCTION

1.1. Anxiety

Anxiety is an aversive emotional state related to perceived future threat[1]. It can be seen as an independent emotion or as a combination of fear together with two or more of the following emotions: guilt, shame, distress, interest-excitement or anger[2]. Anxiety is characterized by a range of somatic, cognitive, emotional, and behavioural components[3]. The experience of anxiety is often accompanied by dread and inner turmoil, which can be focused on a perceived future threat, or an anticipated negatively evaluated future event. There is considerable overlap between anxiety and fear, however fear is an emotional response to an imminent threat, whereas anxiety anticipates a future threat. Fear is more often associated with escape behaviours, autonomic arousal surges in conjunction with fight-flight responses, and thoughts of immediate danger, while anxiety is more often associated with avoidant / cautious behaviours, preparation for future danger and muscle tightness. Panic attacks are a type of fear response which can be associated with anxiety disorders[4]. Fear and anxiety can be viewed as adaptive defense mechanisms – in this context, fear is a response to present threats, while anxiety is an adaptive mechanism, by which potential threats and dangers are predicted, and an emotional response of anxiety drives preparatory strategies aimed at reducing the likelihood of adverse outcomes resulting from potential future threats[5]. Threat detection and prediction are therefore pivotal processes determining whether anxiety plays an adaptive role as a survival mechanism, or becomes a detrimental, maladaptive emotion, which may predict the emergence of anxiety disorders[6].

Anxiety can present with signs and symptoms across the following domains: somatic (e.g. shortness of breath, perspiration, tachycardia, chest pain/discomfort, muscle tension, tremulousness, sensation of abdominal "butterflies", dizziness, feeling faint, numbness/tingling and other manifestations of autonomic arousal); cognitive (impaired concentration and attention, ruminations on anxiety themes); emotional (distress, dread, worry, feeling on edge, apprehension, feeling overwhelmed); and behavioural (avoidance, escape, safety behaviours). Anxiety symptoms are common in the general population, and are experienced by most, if not all people from time to time. Anxiety can however become persistent and cause significant distress associated with impairment of function and reduced quality of life – at which point an anxiety disorder may develop. A distinction between state and trait anxiety was first proposed by Cattell and Scheier in 1961[7]; these concepts were utilised by Spielberger, Gorsuch, and Lushene in constructing the State-Trait Anxiety Inventory (STAI)[8]. State anxiety is defined as transient feelings of anxiety, arousal and tension associated with specific circumstances, conditional on the ongoing perceived presence of specific threats. Trait anxiety is an enduring dispositional tendency towards worry, tension and arousal. In contrast to state anxiety, trait anxiety is not directly observable – but rather inferred from the frequency of observable episodes of state anxiety[1]. From an evolutionary perspective, it may be argued that fear and anxiety mechanisms enabled our evolutionary predecessors to effect a rapid shift of attention away from a current routine task at the first indication of potential danger, such as predator approach[9]; but in the modern environment, we are subjected to a rapidly increasing volume of information carrying potential threat content, delivered by multiple media into every

aspect of our lives – and in this context nearly 1 in 4 of us will experience an anxiety disorder at some point.

1.1.1. Anxiety disorders

Anxiety disorders are the most globally prevalent mental disorders in developing and developed countries[10]. The lifetime prevalence of anxiety disorders in the general population is estimated at 21%, and the 12-month prevalence at 14%[11]: in excess of 60 million people are affected by anxiety disorders in the European Union every year[12]. Anxiety disorders account for almost one-third of the total expenditure for mental illness in the United States, three quarters of this expenditure being due to indirect costs[13]. The cost of direct and indirect expenditure due to anxiety disorders in the European Union exceeded €70 billion in 2010[14]. The diagnosis of anxiety disorders is guided by opertaionalised criteria set out in the International Statistical Classification of Diseases and Related Health Problems (ICD-10)[15] published by the World Health Organisation (WHO), and in the American Psychiatric Association's Diagnostic and Statistical Manual (DSM-5)[4]. In order to confirm a diagnosis of anxiety disorder, one needs to demonstrate that the diagnostic criteria are satisfied by the presence of a specified number of symptoms, for a sufficient duration, associated with significant impairment and distress. The main diagnostic entities in the category of anxiety and related disorders are: Generalised anxiety disorder, panic disorder (with or without agoraphobia), social anxiety disorder (social phobia), post-traumatic stress disorder, obsessive-compulsive disorder, illness-anxiety disorder, separation anxiety disorder, and specific phobia. The 12-month prevalence of anxiety disorders in the general population varies between less than 1% for Obsessive Compulsive Disorder,

approximately 3% for Post Traumatic Stress Disorder, and Generalised Anxiety Disorder, and 6% for specific phobias. The female : male ratio for anxiety disorders as a whole is 2 : 1 across all ages[12]. Anxiety symptoms are also commonly encountered in the context of other disorders, these include other mental disorders (in particular mood disorders) as well as a wide range of physical health disorders. Anxiety symptoms satisfying the diagnostic criteria for an anxiety disorder, which occur in the context of another diagnosed disorder, are termed comorbid anxiety disorders, and occur frequently in the presence of unipolar and bipolar mood disorders, schizophrenia, substance use disorders and physical health disorders[12].

1.1.2. Generalised anxiety disorder (GAD)[4,16]:

GAD was first introduced as a residual diagnostic category for those who did not meet criteria for other anxiety disorders in DSM III[17]. DSM-III-R[18] defined GAD using the core features of chronic and pervasive worry[19]. GAD is currently[4] characterised by difficult to control, excessive anxiety and worry about a number of activities or events, which occurs on most days for at least 6 months. These are associated with at least three of the following symptoms: restlessness/feeling keyed up, being easily fatigued, difficulty concentrating/mind going blank, irritability, muscle tension, sleep disturbance. The anxiety/worry is associated with significant distress and functional impairment. The disturbance is not due to a substance or another medical condition, and is not better explained by another mental disorder. The essential characteristic of GAD is the presence of excessive apprehensive expectation, which is out of proportion to the actual impact and likelihood of the anticipated eventuality.

The 12-month prevalence of GAD is 2.9% in the US general adult population, the lifetime risk in the US is 9%[4]. The European 12-month prevalence is 1.9-5.1% in the general population, and 8% in primary care – indicating that GAD sufferers are frequent users of primary care services. In fact, GAD is the most frequent anxiety disorder presenting in primary care, being present in 22% of those complaining of anxiety symptoms in this setting. [16], but it often goes unrecognised, as most patients present with physical symptoms rather than complaining of anxiety[20]. The prevalence of GAD peaks in middle-age, the female : male ratio is 2 : 1. GAD symptoms tend to persist and fluctuate in severity, with few remissions. The most common comorbidities of GAD are with other anxiety disorders, and unipolar depression. The comorbidity with depression is associated with increased disability days compared to either GAD or depression alone. GAD accounts for 110 million disability days per annum in the US population. GAD is therefore a major cause of long-term morbidity as well as a significant cause of societal economic burden.

Evidence-based acute treatment options for GAD[12] include a range of antidepressants [Selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin-Norepinephrine Reuptake Inhibitors

(SNRIs), Tricyclic Antidepressants (TCAs), and others], pregabalin, benzodiazepines (alprazolam, diazepam, lorazepam), buspirone, antipsychotics (quetiapine, trifluoperazine), and hydroxyzine; as well as non-pharmacological options (CBT and applied relaxation). In the longer term there may be an advantage for CBT over other psychological approaches in relapse prevention[21]. Appropriate treatment of GAD utilising pharmacological and non-pharmacological modalities may improve symptoms of anxiety, as well as prevent the occurrence of comorbid conditions, and reduce the

associated morbidity and economic burden. The functional impairment in GAD can be associated with cognitive impairment related to worry. Worry can be defined as *"a concern over evaluation and failure characterized by expectations of aversive consequences that becomes activated, especially in high trait anxious individuals, in stressful situations that involve evaluative and/or competitive components"*[22]. Worry can cause impairment of cognitive processing efficiency, wasting cognitive resources through maintaining dysfunctional worry, which can be further compounded by the associated efforts to minimize the anxiety – the resulting inefficiencies can disrupt attentional control[23,24].

1.1.2.1. Theoretical models of GAD[19]

1.1.2.1.1. The avoidance model of worry and GAD (AMW)

This model[22,25] regards worry as a thought-based, verbal linguistic activity preventing

the experience of emotional and somatic activation associated with fear-related mental imagery. Borkovec describes worry as an ineffective cognitive attempt to process a perceived threat while avoiding the experience of emotional and somatic confrontation of fear[25]. Worry gets in the way of experiencing the fear, and therefore prevents habituation and extinction[26]. Worry is reinforced by the relief of replacing highly charged imagery, emotional, and somatic experiences with less distressing verbal material; and also by the belief that thinking about potential future threats is a helpful problem-solving strategy.

1.1.1.1.2. The intolerance of uncertainty model (IUM)[27]

The IUM model maintains that people with GAD find uncertain and ambiguous situations difficult to tolerate, and tend to experience chronic worry in response to these situations. This is coupled with the belief that worry is likely to prevent potential negative future events from occurring, or if these do occur, that worry will help to cope with the adverse consequences. The model predicts that people with GAD experience negative problem orientation in that they lack confidence in their ability to solve problems, they tend to see problems as threats, to have pessimistic beliefs about their ability to successfully manage, and they become easily frustrated when trying to solve problems. This constellation of cognitive, emotional and behavioural factors serves to further exacerbate worry and anxiety, creating a 'vicious cycle' of worry and ineffective activity.

1.1.2.1.2.   The metacognitive model (MCM)[28]

The MCM proposes that people with GAD experience two types of worry: *Type 1 worry* is worry about situations that are perceived as potentially threatening – this type of worry is reinforced by positive beliefs about the utility of worry in terms of problem solving, motivation etc. *Type 2 worry* is worry about type 1 worry (and therefore 'meta-worry'). The belief that worry is in itself a reason to be worried differentiates GAD sufferers from non-clinical worriers, and is associated with attempts to avoid the experience of worry (including checking, avoidance, seeking reassurance, suppression and distraction), which in themselves perpetuate and strengthen the worry and anxiety.

1.1.2.1.3.   The emotion dysregulation model (EDM)[29]

EDM draws on emotion theory and on Linehan's[30] conceptualisation of emotional deficits in borderline personality disorder. The model asserts that people with GAD[29].

- Experience emotional hyper-arousal (particularly negative emotions)

- Have poorer insight into their emotions

- Have more negative attitudes about emotions

- Utilise less effective emotional regulation strategies that tend to exacerbate rather than ameliorate their emotional state

Worry plays an important part in this model, as an ineffective emotional coping strategy.

1.1.2.1.4.   The acceptance-based model of GAD (ABM)[31,32]

This model draws on acceptance and commitment therapy (ACT)[33] and on Borkovec's AMW[25]. ABM consists of 4 components:

- internal experiences (thoughts, feelings, or bodily sensations).

- a problematic relationship with internal experiences: A negative reaction to these experiences, and an entanglement with this negative reaction (fusion), which comes to be experienced as a permanent part of oneself.

- experiential avoidance: actively or automatically avoiding negative internal experiences (e.g. worrying about a relatively minor future event in order to avoid more threatening internal content).

- behavioral restriction: The internal avoidance generalises to avoidance of valued external activities.

Roemer and Orsillo suggest that *"individuals with GAD have negative reactions to their own internal experiences, and are motivated to try to avoid these experiences, which they do both behaviorally and cognitively (through repeated engagement in the worry process)"* [31].

1.1.3. Anxiety : Theoretical background

1.1.3.1. Anxiety and anxiety disorders - an evolutionary perspective

1. The evolutionary approach to emotional states and responses in humans and their analogues in other species dates back to Darwin, who described fear responses in this context [34]. Hofer[35] states: *"Of all the clinically important emotions, anxiety may be the one with the closest parallels in other species and with the most ancient evolutionary heritage…To see for a moment how nature put such a state together may give us the perspective from which to find new ways to understand and treat its disorders".* Examples of anxiety analogues in other species range from motile bacteria changing the pattern by which their flagellae rotate in response to noxious stimuli, to separation anxiety in young rats[36]. From an evolutionary perspective, the mechanisms underlying anxiety confer an evolutionary advantage by maximizing fitness (in the Darwinian sense) at the cost of anxiety-related morbidity and suffering[6]. These mechanisms are particularly efficient at offering protection from potential threats at phases of the life-cycle concerned with conceiving, gestating and rearing offsprings[37,38], as well as during childhood, when an evolving pattern of threat-detection biases and related anxieties has been shown to be part of the normal spectrum of child

development[39,40]. There is also evidence that both genders undergo biologically driven changes in threat-detection and risk-avoidance patterns throughout the human life cycle[38]. A range of threat-related responses have been described in higher mammals, these include general anxiety as a way of responding to threats of unclear nature[41] as well as specific subtypes of anxiety responses which evolved in response to particular types of threat: escape (flight); avoidance (pre-flight); aggressive defense; freezing (immobility); and submission. From a clinical perspective, the individual's suffering and impairment are central, and anxiety disorders are therefore a source of considerable morbidity and suffering, as well as an important therapeutic challenge. The 'smoke detector' principle[42], states that *"when the cost of expressing an all-or-none defense is low compared to the potential harm it protects against, the optimal system will express many false alarms"* – from an evolutionary perspective, anxiety disorders that do not impair the ability to rear offspring are a price worth paying for a threat-detection system which confers a survival advantage for these offspring, and therefore false alarms are a valuable trade-off regardless of the attendant personal distress and impairment. There is undoubtedly a degree of discrepancy between what might count as a disorder from the evolutionary vs. the clinical view-points; the evolutionary perspective which sees Darwinian fitness as being of overarching importance, may not be a suitable yardstick for discriminating between what is normal vs. abnormal anxiety[43].

1.1.3.2. Psycho-analytical theories of anxiety

Psychodynamic theory views anxiety as originating from early relationship experiences in which feelings of abandonment, loss, or grief, or wishes to express anger or self-

assertion, were experienced as unacceptable, dangerous, or painful. As a result, these feelings become disavowed, experiencing them is avoided and anxiety symptoms develop[44,45]. Freud in his earlier work[46] conceptualized the 'toxic theory' of anxiety: Anxiety as resulting from repression of undischarged libido[47]. In his later writings, he put forward the 'Signal theory' of anxiety[48]: When the Id generates impulses which are perceived by the Ego as threatening, the Ego responds by signaling anxiety. Defense mechanisms then render the original impulses unconscious – therefore anxiety causes repression. In the *"instincts and their vicissitudes"*[49], Freud describes the symptom of anxiety as a distorted form of the unacceptable instinct, modified in such a way that it becomes less threatening. Structurally[50], Freud viewed a symptom of anxiety as a sub-optimally effective response by ego defense mechanisms to unresolved conflicts between reality and/or the demands of the super ego, and aggressive or sexual impulses generated by the Id. Later psychodynamic models[46] – object relations theory[51] and self psychology[52] - identified a range of conflicts and fears including fear of persecution, separation, disintegration, annihilation, and fusion. In anxiety disorders, anxiety has itself become the psychological symptom.

As for the psychoanalytic formulation of GAD[46], Freud's description of 'anxiety neurosis'[53] emphasises the role of 'fearful expectation' (worrying). Inter-personal relationships commonly underlie this fearful expectation[54], and this may resonate with the association between GAD and insecure attachment, and with past experience of trauma[22,54]. In this context, constant worrying may serve as a defense against trauma recollections, which are more threatening than the experience of worrying is in itself[22].

1.1.3.3. Beck's schema-based theory

Beck first developed the schema-based theory for major depressive disorder[55], and subsequently extended it for anxiety disorders[56]. The theory proposes that *"prepotent maladaptive schematic representations of the self, world and future are activated by matching life experiences"*[57], and as a result, a bias towards processing schema-congruent information leads to an overemphasis on threat-related, negative thoughts, images and interpretations. Structurally, the cognitive model of emotional disorders consists of three cognitive layers[57]: 1. *Schemas*: structurally rigid, absolute and impermeable subjectively biased structural representations of reality. These are based on inferences made from recurrent thoughts, images and biased information processing. 2. *Biased information processing*: anxiety-related schemas lead to selective processing of negative, threat-related information and interferes with processing of schema-incongruent positive information. 3. *Negative automatic thoughts (NATs):* thoughts, images and memories driven by dysfunctional schema and biased cognitive processing, which amplify and perpetuate the anxious state, and feed back into further affirmation and strengthening of the underlying schemas. Developmentally, the theory proposes that dysfunctional self-schemas originate in early adverse experiences such as loss, abandonment, neglect and rejection. These remain dormant as cognitive vulnerabilities, and can be activated by later events and circumstances that can trigger an episode of anxiety. Recurrent activation strengthens the schemas and increase the individual's vulnerability to further, more severe anxiety in response to less severe stress (the diathesis-stress framework)[57]. The theory led to the development of cognitive-behaviour therapy (CBT) and cognitive therapy (CT)[58]; these aim to correct biased cognitive processing and dysfunctional schema activation. Neuroimaging

evidence associates CT with increased activation in frontal cortical areas responsible for top-down information processing, and reduced activation in the amygdalo-hippocampal subcortical region responsible for bottom-up information processing[59-61].

1.1.3.4. Attentional control theory (ACT)

Attentional control is the capacity to direct attention towards task-relevant, and away from task-irrelevant stimuli, and is a key function of the central executive[24,62]. The attentional control theory[24] states that anxiety impairs attention control; it defines anxiety as *"an aversive emotional and motivational state occurring in threatening circumstances"*, and states that the level of current (state) anxiety is determined by individual disposition (trait anxiety) and the level of situational stress[63]. State anxiety is *"a state in which an individual is unable to instigate a clear pattern of behavior to remove or alter the event/object/interpretation that is threatening an existing goal"*[64].

1.1.3.5. The processing efficiency theory

The attentional control theory is an update on the **processing efficiency theory**[65]. The processing efficiency theory distinguishes between 'effectiveness' (defined as the quality of behavioural outcomes e.g. task accuracy) and 'efficiency' (which reflects effectiveness in relation to the effort and resources allocated to the task). The model predicts that anxiety will impair efficiency more than it impairs effectiveness (as effectiveness can be improved by increased resource allocation). Two assumptions underpin the processing efficiency theory:

- Worry is the state-anxiety component responsible for the effects of anxiety on task efficiency and effectiveness. Worry is triggered

(particularly in high anxiety trait individuals) in stressful situations. Worry affects performance in two ways: 1. Worry consumes the limited capacity of working memory by occupying it with worry-related cognitions – and therefore impairs performance. 2. Worry increases effort and cognitive resource utilization to compensate for the effects of anxiety. While additional resources are available, net effectiveness can be maintained at the cost of reduced efficiency.

- The effects of anxiety on working memory are mainly centred on the executive control component of working memory. Working memory can be seen as composed of four components[66]: 1. Phonological loop – auditory information; 2. Visuospatial sketchpad – visual and spatial information; 3. Executive control - concerned with information processing and storage; and 4. Episodic buffer – concerned with transfer of information to long-term memory. Therefore, the effects of anxiety will be more pronounced when tasks are particularly challenging in terms of information storage and processing.

The processing efficiency theory has four main theoretical limitations in terms of precision, explanatory power, and scope[24]:

- It fails to specify which of the components of the central executive are affected by anxiety[67]: Task switching, Sub-task planning, or time & place coding of working memory information.

- It does not address the tendency of anxious individuals to be more impaired by distracting stimuli, compared to less anxious individuals.

- It does not address emotional valence effects of stimuli, there is evidence that anxiety-related impairment is particularly associated with threat-related stimuli compared to neutral stimuli[68].

- It does not address situations in which anxious individuals out-preform less anxious individuals.

1.1.3.5.1. Attentional Control Theory: Assumptions

- Anxiety impairs attentional control by shifting attention away from a current task, towards threat-related stimuli, which can be internal (e.g. worrisome thoughts) or external (threat-related distractors). This widens the scope of anxiety effects from worry (as in the processing efficiency theory) to anxiety related to either internal or external threat-stimuli.

- The presence of significant levels of worry might not reduce task-effectiveness, due to increased utilisation of cognitive resources and effort. Performance efficiency is reduced, but effectiveness is maintained.

- Anxiety also impairs attentional control in the absence of threat-related stimuli. The rationale is that when a non-specific threat is sensed, a safer strategy is to disperse attention widely, rather than maintain a narrow task-related focus.

- Anxiety disturbs the balance between two attentional systems[69,70]: 1. Top-down, goal-directed system; and 2. Bottom-up, stimulus-driven system. Anxiety shifts the attention control from the goal-oriented top-down system, towards the stimulus-driven, bottom-up system.

1.1.3.5.2. Anxiety impairs attentional control through effects on inhibition, task-shifting, and updating control functions

Miyake et al. identified three central executive basic control functions [71]:

- Inhibition: Attentional control is used to prevent the allocation of attentional resources to task-irrelevant stimuli and responses

- Shifting: Attentional control is actively used to shift attentional resource allocation between task-relevant stimuli and responses.

- Updating: Re-assessing and monitoring of working memory representations.

The attentional control theory states that anxiety reduces processing efficiency by impairing attentional control, particularly when threat-related distractors are present. Anxiety is associated with impaired function of all three central executive control functions: inhibition[23,72], with increased distractibility[73,74], shifting[75,76] and updating[72]. Anxiety moves the overall balance of cognitive/attentional resource allocation away from task-relevant, towards task-irrelevant stimuli through effects on the task shifting, inhibition, and updating functions of the central executive. Inhibition becomes progressively impaired as the intensity of task demands on the central executive and on working memory capacity increase[77].

1.1.3.6. Neuro-cognitive models of anxiety:

Understanding of the neurocognitive underpinnings of fear and anxiety is informed by a combination of findings from animal and human research[9]. Fear, the physiological and behavioural response to explicitly threatening stimuli, has been extensively studied in animals using Pavlovian models of fear conditioning. Anxiety is triggered by less

specific, or more implicit cues, and is activated when the certainty of threat is less clear; it manifests as worry and arousal, which are less specific than fear responses. Anxiety is therefore more difficult to study in animal models. Anxious individuals display increased attentional capture by threat-related stimuli, and tend to interpret ambiguous stimuli as threat-related[9], these biases may underlie the development and maintenance of anxiety[78,79]. Neuroimaging offers opportunities for assessing the neurocognitive substrates of human anxiety, and comparing them to what we know about conditioned fear responses in animals. The evidence suggests that amygdalo-prefrontal circuits are important in identifying the emotional salience of stimuli, and in exerting top-down attentional control. The amygdalo-prefrontal dysfunction in anxiety involves two components[9]: 1. Threat-related hyper-activation of the amygdala[80], resulting in over-attribution of threat-related salience to stimuli, which maintains and strengthens attentional, associative and interpretive processing biases. 2. Deficits in the recruitment of prefrontal control mechanisms that regulate attention and reduce distractibility [81,82]. The finding that trait anxiety in itself may be associated with impaired attentional control even in the absence of threat-related stimuli giving rise to current state anxiety[83], may explain the cognitive difficulties that anxious individuals encounter in performing tasks that place demands on cognitive/attentional resources[65,84,85]. The DLPFC is involved in sustaining attention on current tasks[86], and in shifting attention in response to changes in competing processing demands[87]. Interestingly (and somewhat counter-intuitively), active recruitment of attentional resources is particularly important when the task at hand involves lower processing demands or perceptual load (the load theory of selective attention[88]) . The explanation may be that when the task at hand involves high processing demands or perceptual

load, all available attentional resources are already committed to the task, and therefore the processing of competing distractors does not progress as far as entering into working memory or selecting appropriate response. However, when the task at hand is only partially occupying the available attentional resources, competing distractors are allowed to compete for processing resources, and extra attentional control needs to be recruited in order to prevent distraction. When the task at hand involves a high perceptual load, distractor processing is diminished through early selection of distractor stimuli to discard, whereas when the task involves a high cognitive control frontal load, distractor processing is enhanced as stimulus selection is performed later in the cognitive process[89]. Bishop (2009) demonstrated that subjects with high trait anxiety had impaired DLPFC attentional control resource recruitment under conditions of low perceptual load even when state anxiety was controlled for[83]. This predicts that individuals with high trait anxiety will have more pronounced attentional control impairment in situations when their attentional resources are not fully committed to the task at hand. These findings seem to contrast with the attentional control theory (ACT) prediction that prefrontal attentional control mechanisms are compromised particularly when processing resources are stretched. ACT predicts that individuals with anxiety will have *increased* prefrontal resource utilisation (reduced efficiency) in order to maintain effectiveness (accuracy) to compensate for their increased need for countering attentional processing biases – whereas Bishop demonstrated that individuals with high trait anxiety have *reduced* prefrontal resource allocation due to DLPFC recruitment deficits at times when the task at hand does not present high resource demands[83]. Bishop found that when performing tasks requiring high executive demand and perceptual load, there were no

significant differences between high vs. low trait anxiety individuals in their ability to recruit DLPFC resources or in their task accuracy. This may indicate that high trait anxiety is associated with reduced recruitment of DLPFC attentional control resources when the task at hand does not require continuously complete commitment of attentional resources. When the task does not fully absorb attentional resources, salient distractors may cause processing difficulties due to inefficient management of their competing attentional demands. This aspect of trait anxiety may therefore represent more of a processing style rather than a processing deficit – indeed it presents some potential evolutionary advantages in terms of allocating 'spare' cognitive/attentional capacity for maintaining higher threat awareness. It may also be possible to modify this attentional style by helping high trait anxiety individuals to improve their skills in maintaining attentional focus (concentration) at times when their attention is not absorbed in cognitively demanding tasks – mindfulness training may be a conceptually appealing option for achieving this[83].

1.1.3.7. Three attentional networks model

Attention is a complex phenomenon, made-up of several attentional components[90]. A number of sub-cortical and cortical networks interact with each other, and give rise to the group of processes underpinning attention[91]. In 1971 Posner and Boies put forward an early version of a three-network model[70,92,93]. This model maintains that there exist three demarcated attentional networks, each with its own structural and functional characteristics. Despite having its origins prior to the availability of extensive neuroimaging data, modern imaging data lends further support to this model[94-96].

These three networks can be viewed as aspects of an attentional organ system, incorporating histological, neuroanatomical, and functional components[97]. Different three-network models have been proposed over time[98,99], describing parallel, but not identical entities with different names[91]. The three attentional networks proposed by these models are currently termed: 'alerting', 'orienting', and 'executive'[100]. The **alerting attentional network** modulates alertness, which can be defined as the capacity to muster and sustain impending stimulus response-readiness[91,92], or as readiness for receiving information and responding to it[101]. Alertness can be divided into intrinsic (non-specific, endogenous, generalised level of arousal), and phasic (exogenous, task-related) components[102,103]. The alerting system has anatomical associations with right frontal and parietal areas[104], and with the CNS noradrenergic system[100,105,106]. There is an additional layer of complexity associated with the interaction between phasic (task-related) alertness, and intrinsic arousal: arousal is mediated by multiple systems[107] including right frontal networks (sub-cortical & cortical) coordinated by the anterior cingulate cortex[108]; these include frontal, thalamic, brainstem, inferior parietal, and anterior cingulate cortex structures[90]. Task-specific alerting may influence arousal levels via left hemisphere executive networks, and the right dorsolateral prefrontal cortex[91,100], as well as the thalamus, and the superior and ventrolateral frontal gyrus[102]. The **orienting attentional network** is the most thoroughly understood attentional network[91]. Orienting (also referred to as selection or scanning) is defined as the capacity to separate particular items or strands of information out of the totality of available sensory inputs[91]. The orienting attentional system mobilises attention towards specific stimuli. Top-down (endogenous) orienting is driven by executive processes, while bottom-up (exogenous) orienting involves

automatic capture of attention by external stimuli[109]. Orientation can also be subdivided into overt and covert orientation (e.g. with or without eye movement) [110]. The concept of re-orienting refers to the alteration of attentional focus and direction in response to unexpected stimuli[111]. Areas associated with orienting functions include the superior parietal and temporal lobes, temporoparietal junction, and frontal eye-fields[91]. There is evidence for 2 sub-networks: the first is top-down, dorsal network, directing attention towards goal-directed stimuli - it includes areas within the superior frontal and intra-parietal cortices. The second sub network is a bottom-up network, which includes areas within the inferior frontal and temporoparietal cortices. This right, lateral ventral system can act as a stimulus-driven cut-off system, re-orienting attention towards salient, unexpected stimuli[69].   The **executive attentional network** is responsible for top-down, higher-level processes involving resolution of conflicts between competing computations or stimuli, and allocating attentional capacity to concurrently presenting stimuli and/or active regions. Executive network functions may include making decisions, detecting errors, cognitive and emotional regulation, suppression of habitual responses, and navigating danger or difficulty [87,91,112]. Anatomical regions associated with executive functions include the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex, locus coeruleus, and the ventral tegmental area[91,95,113]. While the executive system is responsible for top-down attentional control, the orienting and alerting systems have both exogenous (bottom-up, stimulus-driven), and endogenous (top-down, goal-oriented) components. Trait anxiety may be related to top-down processes that may be associated with impaired executive, and endogenous alerting/orienting attentional systems. Trait anxiety may also be related to more sensitive bottom-up processes. Anxiety-related attentional biases can be

associated with cognitive mechanisms affecting trait (rather than state) anxiety, and therefore can be demonstrated by affectively-neutral tasks, in low anxiety conditions[114,115]. Anxiety-related attentional biases may be related to a wider dysregulation of attentional control, impairing global attentional processing[62]. Neuroimaging evidence supporting this include findings of an association between trait anxiety and impoverished recruitment of prefrontal distractor inhibition attentional control mechanisms, which persists in the absence of threat-related stimuli even when state anxiety is controlled for[83].

1.1.3.8. Experimental tasks

Attentional control and attentional bias in anxiety have been explored in both clinical and non-clinical populations using a range of experimental tasks. A meta-analysis of eye-tracking studies of attentional bias in anxiety[116] (N=33, n=1579) found that compared to non-anxious controls, anxious subjects demonstrated increased vigilance for threat (Hedges' g = 0.47 [95% CI: 0.25-0.69]) during free viewing and visual search, and more difficult threat-disengagement in visual search tasks only (Hedges' g = 0.54 [95% CI: 0.17-0.92]). A meta-analysis of threat-related attentional biases in anxiety (N=172, n=4031)[117] showed a consistent attentional bias across a range of experimental paradigms and conditions (Cohen's d = 0.45 [95% CI: 0.40-0.49]). Both conscious and non-conscious threat-related stimuli were associated with attentional bias. The bias was consistent across clinical diagnostic categories, and different age groups, and extended to non-clinical high anxiety subjects but not to non-anxious subjects.

1.1.3.8.1.   The anti-saccade task

The anti-saccade task (Figure 1.1), first described by Hallett [118] has emerged as an important tool for investigating subjects' ability to flexibly control behaviour[119], with good test-retest reliability[120]. Ettinger[121] describes the anti-saccade task as *"a model of the conflict between an unwanted reflexive response (which must be inhibited) and a complex volitional response (which must be generated)"*. The task presents a central fixation marker followed by an abrupt appearance of a target in the periphery of the subject's visual field, and requires the subjects to generate an eye movement in the opposite direction (anti-saccade) to the target, towards a position mirroring that of the target. The task requires a two-step process: 1. suppression of the automatic pro-saccade (the tendency to look towards the target), and 2. generating a voluntary anti-saccade towards the position mirroring the presented target. The task decouples stimulus location from saccade goal, and requires inversion of the stimulus vector to generate the saccade vector. Anti-saccade performance can be compared to performance on a task requiring the subject to look towards the presented target (pro-saccade task), to provide information on attentional control functions of inhibition, shifting, and updating.   Monkeys can be trained to perform a version of the task whereby anti-saccade and pro-saccade tasks are differentiated by the colour of the presented target, the behaviour patterns produced by monkeys in this task are qualitatively similar to those produced by humans. The general patterns observed in these tasks[119] are: 1. correct anti-saccades are generated later than correct pro-saccades; 2. direction errors are mostly on anti-saccades, and are generated earlier than correct movements; 3. removing the fixation marker before presenting the target is associated with reduced latency and increased anti-saccade errors; 4. both pro-

saccades and anti-saccades demonstrate bimodal distributions – a low-latency 'express saccade' (a prepotent response) in which the target stimulus is translated directly into a pro-saccade (which generates fast errors on the anti-saccade task); and a delayed saccade (volitional response) reflecting the time required for computation (which is greater for correct anti-saccades than for correct pro-saccades due to the increased complexity of the computation required). Correct performance on the anti-saccade task requires top-down control to prevent express-saccade related directional errors.

The anti-saccade task provides two performance measures[122]: 1. *Performance effectiveness* – anti-saccade accuracy rate; 2. *Performance efficiency* – correct saccade latency. Compared to low-anxious (LA) subjects, high anxious (HA) subjects have longer correct anti-saccade (but not pro-saccade) latencies – suggesting that the anxiety-related deficit is in the inhibitory component of attentional control, leading to reduced efficiency; there were no significant differences between HA and LA subjects in error rate – suggesting that anxiety reduces efficiency but not effectiveness [75,123]. Subjecting healthy participants to severe threatening-stimuli version of the anti-saccade task (using aversive images from the International Affective Picture Set) resulted in an elevated error rate (reduced effectiveness) in HA vs. LA participants[124] – which may reflect the additional cognitive processing required to over-ride the attentional bias towards threatening stimuli in HA participants. HA participants required to randomly shift between pro- and anti-saccade tasks (on a mixed anti-saccade task), showed reduced ability to shift attentional resources in response to task changes, when compared to LA participants[75]. In a study of anti-saccade task under incentivised vs. non-incentivised vs. punished conditions in adolescents with GAD compared to healthy

adolescents and adolescents with MDD[125], the inhibitory efficiency of the GAD group in incentivised vs. non-incentivised trials was reduced when compared to the healthy group. A study comparing the anti-saccade task performance of adolescents with various anxiety disorders to healthy controls[126] found enhanced inhibitory control following exposure to threat cues (fear faces) only in the anxiety disorder group, and following exposure to positive cues (happy faces) only in healthy controls. Use of the 7.5% CO_2 paradigm to induce anxiety in healthy individuals is associated with longer anti-saccade latencies (reduced efficiency) and with increased anti-saccade errors towards threat-related stimuli (reduced effectiveness)[127]. An fMRI study of the anti-saccade task in healthy volunteers[121] aimed to identify the activation pattern associated with the two main cognitive components of the task: inhibition of the pro-saccadic reflex, and generation of the volitional anti-saccade. The study found that ventrolateral and dorsolateral prefrontal cortical areas were activated throughout both components of the task, and concluded that these areas were involved in executive task coordination. The drawback of this study was the 24-36 second duration of each trial, which may have introduced a delay in which other cognitive processes could have confounded the results. A study of evoked response potentials (ERPs) during pro-saccade and anti-saccade found that compared to LA individuals, HA individuals had longer anti-saccade latencies, and lower ERP activity, at frontocentral and central recording sites, than low anxious individuals immediately prior to correct anti-saccade trials. The authors concluded that this was evidence of anxiety-related reduced recruitment of frontal top-down attentional control resources needed for suppression of reflexive pro-saccade[128].
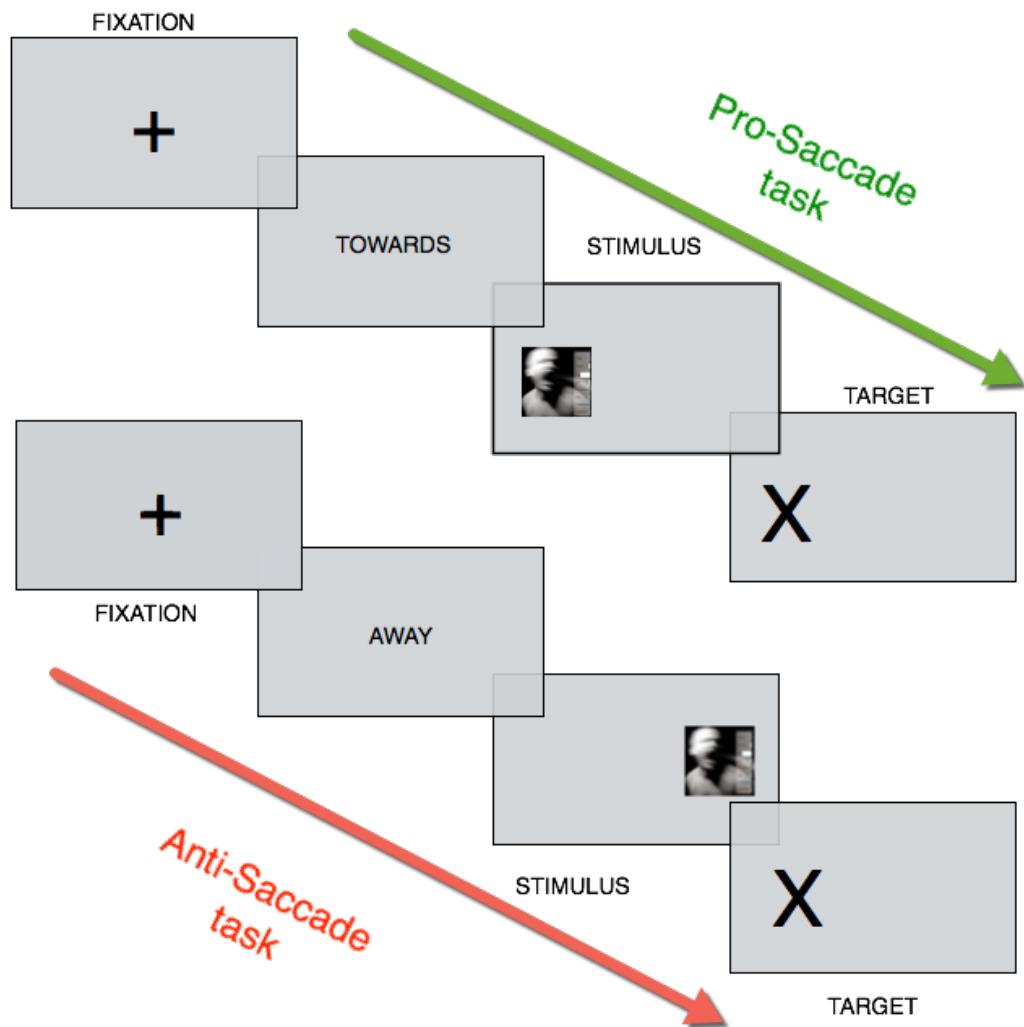
Figure 1.1. Pro-saccade and anti-saccade tasks: Subjects fixate on a marker, and are then instructed to look either towards or away from a stimulus (adapted from Ansari et al. 2008[129])

1.1.3.8.2. The Attention Network Test (ANT)

The Attention Network Test (ANT) is a computerised reaction time test first described by Fan and colleagues in 2002[100]. The ANT uses a combined cued reaction time[109] and

flanker tasks[130] to independently measure the performance (Response Time and Error Rate) of the alerting, orienting and executive attention networks. The original version of ANT repeatedly invites subjects to respond to a central arrow pointing either right or left; the arrow may be flanked by four additional distractor arrows – these may point in the same direction as the central arrow (congruent condition), or in the opposite direction (incongruent condition). Subjects are intermittently cued by temporal and/or spatial visual stimuli – providing alerting and orienting cues. Several modified versions of the ANT have been developed – allowing for instance to differentially assess re-orienting and orienting by contrasting reaction times and accuracy between validly cued stimuli, and stimuli presented following false (invalid) spatial cues[131]; another modified version allows the arrows to be presented to the right or left of the fixation point[132]. Subjects undertaking the ANT are presented with a series of visual stimuli (See Figure 1.2) and tasked with responding as fast as possible by indicating whether the central arrow is pointing to the right or to the left. The arrows are preceded by "+" cues – these cues can be central, double, absent (no-cue) or spatial. Calculations of effects are conducted by subtracting Mean Response Times (RT).

- Alerting effect $\qquad$ = Mean RT $_{(No\ cue\ trials)}$ − Mean RT $_{(Double\ cue\ trials)}$

- Orienting effect $\qquad$ = Mean RT $_{(Centre\ cue\ trials)}$ − Mean RT $_{(Spatial\ cue\ trials)}$

- Executive Control effect $\qquad$ = Mean RT $_{(Incongruent\ trials)}$ − Mean RT $_{(Congruent\ trials)}$

Higher executive control scores suggest worse executive control performance, as the mean RT difference between the simpler congruous and more challenging incongruous trials increases. Higher Alerting and Orienting scores reflect improved performance of these networks i.e. RT's are faster (smaller) on double cue relative to no cue trials, and on spatial relative to centre cue trials .

The relationship between anxiety and attention has long been recognised[133]. Attentional abnormalities associated with anxiety include hypervigilance[134], and attentional bias highlighting anxiety-related stimuli[135]. The spectrum of anxiety is often subdivided into state and trait anxiety. The effects of these subtypes of anxiety on attention have been proposed to be different[136] – state anxiety enhances the threat valence of a stimulus, whereas trait anxiety causes attention to be directed consistently towards potential sources of threat. State anxiety is triggered by situational factors, so is likely to be associated with bottom-up processes, whereas trait anxiety is related to personality factors and therefore likely to be more associated with top-down processes[73]. There is some evidence demonstrating that trait anxiety is associated with reduced executive control performance on the ANT: Pancheco-Unguetti and colleagues used a modified form of the ANT (ANT Interactions ANT-I) to test subjects with high vs. low trait anxiety scores[73]. In the first study, high trait anxiety subjects demonstrated significant deficiencies in executive control (but not in alerting or orienting) network performance. The second study found an association between state anxiety and increased performance of the orienting and alerting networks, but no difference in executive network performance. Pancheco-Unguetti and colleagues[74] compared performance on the ANT-I between patients with anxiety disorders and healthy controls. Anxiety disorders were associated with executive attentional network dysfunction and with reduced efficiency in attentional disengagement from invalid cues – including emotionally neutral cues. State anxiety is positively correlated with increased alerting performance on the ANT[137]. Han and colleagues showed that

adolescents with comorbid depression and anxiety disorder demonstrated a faster orienting response on the ANT when compared to depressed adolescents without comorbid anxiety disorder[138]. These findings may reflect a combination of trait/state anxiety-related impaired executive network function, and increased vigilance related to state anxiety.
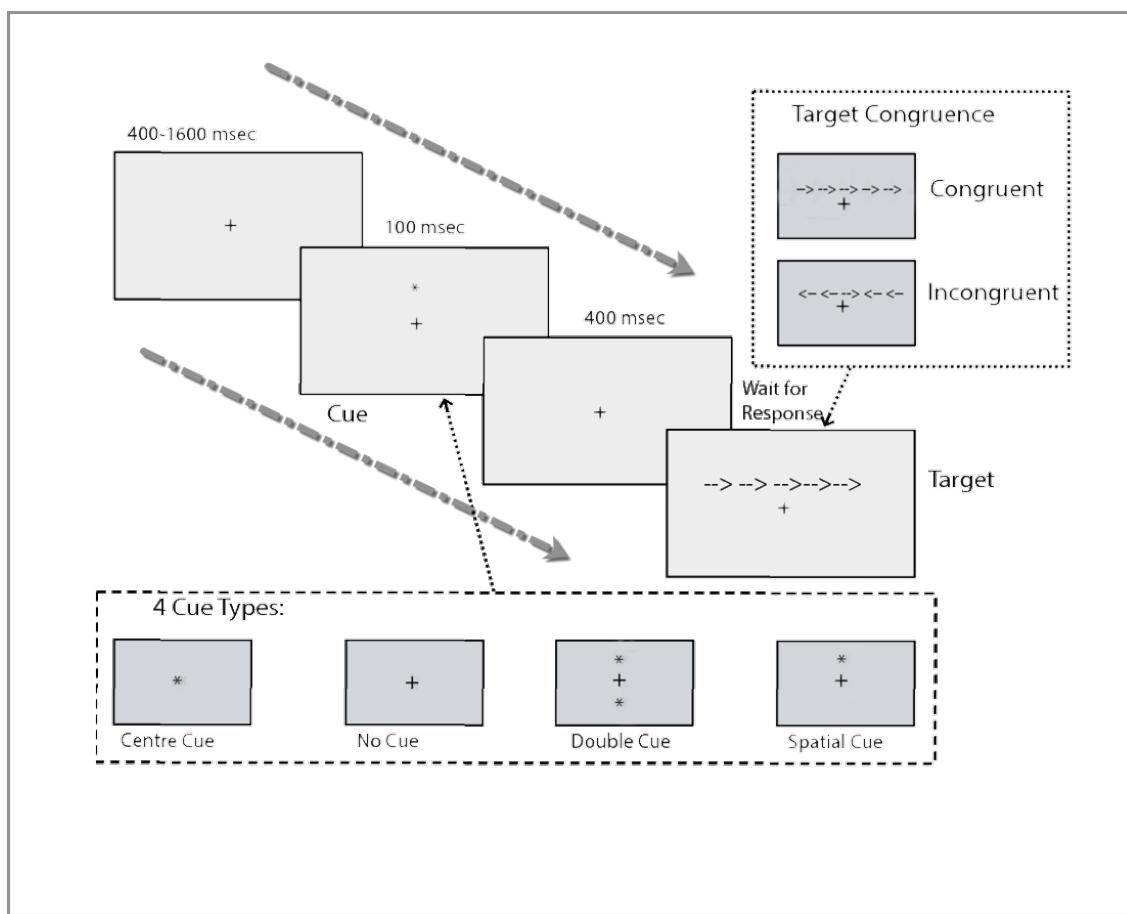
Figure 1.2 The Attention Network Test (adapted from Garner et al, 2012)[127]

1.2. Evaluating new treatments in anxiety disorders[139]

Existing treatments for anxiety disorders have significant limitations in terms of efficacy, tolerability, potential for dependence, cost, and availability[12,140-142]. Developing new treatments for anxiety disorders is therefore necessary. Advances in

neuroimaging, large-scale genotyping studies, and our understanding of brain processes underlying anxiety have yielded a range of candidate treatments for anxiety. However, few new treatments (particularly new pharmacological treatment) have made the transition to clinical use. The reasons for this are complex, and the costs involved in developing new treatments only to have them fail before reaching clinical practice are prohibitive. Robust, validated healthy subject models of anxiety can help in assessing the potential utility of new treatments for anxiety, and thus may be a useful method for screening new treatments and managing the risks of transferring treatments from the laboratory to a clinical setting. Experimental models of anxiety can facilitate exploration of the cognitive, emotional, somatic, and behavioural aspects of anxiety, and clarify the mechanisms by which treatments for anxiety work[143]. Such models are required to be safe, acceptable, reliable, translational, easy to use, repeatable, and able to produce both subjective and objective outcome measures [139]. Broadly speaking, these models can induce anxiety in 3 ways: Pharmacologically – by administering anxiogenic substances, physiologically – by inducing pain, or shortness of breath; or psychologically – exposure to anxiety provoking situations (e.g. public speaking)[139].

1.2.1. 7.5% CO2 model

Inhalation of air mixtures containing elevated concentrations of carbon dioxide (CO_2) has long been known to induce symptoms of anxiety and panic[144]. CO_2-enriched air has been utilised for anxiety induction in healthy subjects[145,146], with good test-retest reliability[147]. In animals, breathing 10% CO_2 is associated with fear-related behaviours, and is thought to be mediated via chemosensors in the amygdala which are sensitive to

CO_2 concentration and pH[148]. In humans, inhalation of concentrations of CO_2 greater than 7.5% is associated with a range of subjective and objective manifestations of anxiety. Physiologically, there are signs of autonomic arousal including elevated blood pressure, pulse rate and sweating. Healthy subjects describe feelings of anxiety, tension and fear[149-151]. Inhalation of 7.5% CO_2 for 20 minutes is associated with emergence of anxiety symptoms in patients diagnosed with GAD[152]. Patients with panic disorder experience panic following a single inhalation of 35% CO_2[153-156]. Bailey and colleagues examined the 7.5% CO_2 model as a potential experimental model of GAD in two separate studies of healthy volunteers: In study 1 subjects were given a single dose of lorazepam, and in study 2 participants were treated with 21 days of the SSRI paroxetine. The authors concluded that the 7.5% CO_2 model is sensitive to a treatment with efficacy in GAD, and that this supported the model's utility as an experimental model of GAD in healthy volunteers[149]. This conclusion is further supported by evidence from GAD patients in whom the clinical picture of GAD is reproduced when exposed to a 7.5% CO_2 challenge[152]. The 7.5% CO_2 model of anxiety in healthy subjects has been shown to be particularly sensitive to the effects of benzodiazepines and a corticotrophin releasing factor (CRF1) antagonist [157], less sensitive to the effects of SSRIs, and not sensitive to venlafaxine and pregabalin[139] – this may be related to dosing and timing issues in the studies, but may also relate to limitations of the model. To date the model has not been used to test psychological interventions in GAD. Healthy subjects exposed to the 7.5% CO_2 model showed significantly reduced accuracy rate on the anti-saccade task towards threat-related picture cues[143], this is consistent with findings in HA vs. LA healthy subjects exposed to threat-related pictures[124], and with the finding that patients with GAD show increased orientation towards threat-related

stimuli in a modified probe detection task[158]. Healthy subjects exposed to the 7.5% CO_2 model showed significantly increased alerting and orienting attentional network performance (hypervigilance) on the ANT[127]. Compared to breathing normal air, exposure to 7.5% CO_2 was associated with increased autonomic arousal and reported anxiety. This effect was particularly marked in high trait anxiety subjects. The 7.5% CO_2 model represents a validated experimental model of GAD that can be used for the evaluation of treatments for GAD.

1.3. Mindfulness

Mindfulness-based applications have over recent decades become increasingly prominent across a range of settings, including health, education, and neuroscience. Since the late 1990s, there has been an exponential increase in the number of mindfulness-related research publications in the scientific literature [159]. The Buddhist context, within which mindfulness was originally described, is focused on eliminating suffering (*Dukkha*). Indeed, mindfulness is presented as one of the components of the Buddha's eight-fold path - the solution to the problem of suffering[160]. The Buddhist[161] (and indeed the pre-Buddhist[162]) origins of mindfulness as a concept, discipline, and practice, are being adapted to fit a modern, scientific, and largely secular approach to the alleviation of suffering in the context of western health-care delivery[163].

1.3.1. The Buddhist origins of mindfulness

The roots of mindfulness date back to the teachings of the Buddha, who lived in northern India in the 5th Century BC. The Buddha developed his teachings, known as the *Dhamma* (Sanskrit Dharma), as a set of principles and practices aimed at alleviating

human suffering. The earliest, and arguably most well known formulation of the Buddha's understanding of human suffering is encapsulated by the four Noble Truths: 1. The Truth of suffering: all phenomena are ultimately unsatisfactory; 2. The Truth that craving and aversion are the origins of suffering; 3. The cessation of craving and aversion results in the cessation of suffering; and 4.The Noble Eightfold path is the way to cessation of craving and aversion (and therefore the end of suffering)[164]. The *Dhamma* underwent many transformations over the ensuing 25 Centuries, spreading east from India to China, Southeast Asia, Japan, and on to North America and Europe[161]. The concept of mindfulness (Pali: Pali; Sanskrit: *smṛti*) is integrated into every aspect of Buddhist theory and practice – in the Pali canon, the Buddha is quoted as saying "*But mindfulness, bhikkhus (monks), I say is always useful*" (Samyutta Nikaya V, 46, 53). Indeed, the early Buddhist view of mindfulness is multi-faceted, and permeates through various facets of the *Dhamma* (Pali term denoting reality, law, teachings) as presented in *suttas* (Sanskrit sutras, Buddhist scripture) of the Pali canon[162]:

- Mindfulness as the root of a sense of conscience: Underpinning ethical conduct (Sila). The precept-based prohibition against the use of alcohol and other mind-altering drugs is rooted in the rationale that these substances undermine mindfulness and therefore dissolve the foundation for living a moral life.

- Mindfulness as memory: Pointing to the ability to remember the teachings, which in the Vedic tradition were passed through the generations by oral recitation. *Sati* is the faculty that enables a recollection and intellectual understanding of the Dhamma (the Buddhist teaching), with a view to applying them to life as it unfolds from moment to moment.

- Mindfulness as the guardian of the six sense doors[165]: Buddhist psychology views the mind as having six sense 'doors' – input gateways connecting senses with corresponding sense objects. These include the five conventional sense modalities: visual, auditory, olfactory, gustatory, and tactile (including internal body proprioception); and in addition also the modality of mind, sensing mental objects such as thoughts, and feelings. Mindfulness is seen as a guardian, preventing the mind from being swept away by the incoming stream of sensory events.

- Mindfulness constantly and repeatedly re-collects and re-members the mind, to enable one to be rooted in present reality.

- Mindfulness as the seventh component of the Buddhist eight-fold path to liberation[166]: right mindfulness (*sammā-sati)* – practitioners are encouraged to cultivate awareness of body and mind.

- Mindfulness as applied in meditation[167] (and to meditation), enabling discernment of states of mind, and identification of ever more refined stages of mental development and higher consciousness (*Samadhi* – the one-pointed, unified mind). Mindfulness underpins the mind's development along the whole spiritual journey.

- Mindfulness as liberation: The realised *Arhat* (one who has achieved liberation) is described as 'ever mindful'[162].

In contrast to this wide-ranging scope of mindfulness, modern Buddhist literature in the west predominantly emphasizes the aspects of mindfulness as closely related to

insight (Vipassana), as described in the *Theravada Satipatthana Sutta* (the *Sutta* of the four foundations of mindfulness)[168]. This *Sutta*, referenced by most modern descriptions of Buddhist insight meditation, has achieved prominence as an authentic exposition of the Buddha's teachings on mindfulness meditation[169], and is described as "*the most important sutta in the entire Pali canon*"[170]. The *Sutta* begins by describing its purpose and methods[161]:

> "*Monks, this is the one-way path for the purification of beings, for the*
>
> *overcoming of sorrow and lamentation, for the passing away of pain and*
>
> *displeasure, for the achievement of the method, for the realization of nibbana,*
>
> *that is, the four establishments of mindfulness. What four? Here, a monk dwells*
>
> *contemplating the body in the body . . . feelings in feelings . . . mind in mind*
>
> *. . . phenomena in phenomena, ardent, clearly comprehending, mindful, having*
>
> *removed covetousness and displeasure in regard to the world. This, monks, is*
>
> *the one-way path for the purification of beings . . . for the realization of nibbana,*
>
> *that is, the four establishments of mindfulness.*"
>
> DN 22.1 (II 290; LDB 335). MN 10.1 (I 55; MLDB 145).

The aim of the practice is the eradication of suffering and the attainment of *Nibbana* (Sanskrit: *Nirvana*) – a state of extinction or cooling of the passions. The method involves establishing the four foundations of mindfulness: body, feelings, mind, and phenomena. Mindfulness in this context involves contemplation (*anupassana*: closely seeing) and clear-comprehension (*sampajanna*). Mindfulness (*Sati*) can be seen as lucid awareness of the phenomenal field, whereas Clear-comprehension (*Sampajanna*) adds

a cognitive component whereby the meditator is not only aware of present reality, but also intuitively comprehends the nature of observed phenomena within the context of the *Dhamma*[161]. Seen from this angle, *Sati* is present at the earlier stages of mental development, and Sampajanna is developed later as *Sati* becomes more stable and established. It is interesting to note that whereas the modern uses of mindfulness stress aspects of bare-attention, and non-judging, *Sampajanna* involves discernment and distinguishing between skillful and unskillful, and between wholesome and unwholesome. *Nayanaponika Thera* states that bare awareness is at play when the practice involves a purely observational, receptive frame of mind, whereas practices involving action also require the quality of *Sampajanna*[171]. The tension that arises between these understandings can be resolved by conceptualizing bare-awareness as a procedural instruction for practitioners seeking to develop mindfulness, whereas the discerning comprehension aspect of mindfulness develops as mindfulness matures and stabilizes. Mindfulness training can also be directed at developing one-pointed calm concentration (*Samatha*), or at cultivating an open, choice-less, inclusive mind that can lead to insight (*Vipassana*). Theravada traditions approached these sequentially, first stabilizing the mind by practicing *Samatha* to develop calm concentration, and subsequently moving on to practice observation of the mind in action in order to attain insight. A potential drawback of this approach is the risk of becoming attached to highly concentrated mind states (*Jhannas*), which can be experienced as pleasurable, quietist, or disconnected from the challenges of daily life. This ultimately leads to a dead-end and cannot of itself provide a lasting transformative relationship with life, suffering and liberation. Later approaches, for example the *Chan* (Chinese Zen) practice of silent

illumination, developed strategies enabling practitioners to calm the mind while simultaneously developing insight.

1.3.2. Contemporary approaches to mindfulness within healthcare settings

Since the late 20th Century, mindfulness has become increasingly recognised as a treatment option for a range of symptoms and disorders. Two main modalities of mindfulness-based interventions are described in the literature: Mindfulness Based Stress Reduction (MBSR), and Mindfulness Based Cognitive Therapy (MBCT). In addition, other therapeutic approaches are linked to mindfulness-based ideas, or employ meditation as part of the intervention – these include among others: Dialectical Behavior Therapy (DBT)[30], and Commitment Therapy (ACT)[33], and Relapse Prevention (RP)

1.3.2.1. Mindfulness Based Stress Reduction (MBSR)

Jon Kabat-Zinn, a pioneer of mindfulness approaches in healthcare, utilised his training in meditation to develop Mindfulness Based Stress Reduction (MBSR), first described in his book "Full Catastrophe Living"[172]. MBSR draws on teachings of the Buddhist traditions (Dharma), combining them with modern medical science. The Vietnamese Zen master Thich Nhat Hanh described MBSR as *"a door from the Dharma to the world as well as from the world to the Dharma"*[163]. MBSR is a complex intervention, aimed at reducing the suffering of people with a range of medical disorders and symptoms for which no definitive medical treatment has been found. MBSR is delivered in a facilitated group setting over eight weekly sessions, combining sitting meditation, body scan meditation, basic yoga, and individual and group dialogue and inquiry[173].

Participants are supported in developing a daily mindfulness practice, and are encouraged to continue practicing daily after the course ends.

1.3.2.2.   Mindfulness Based Cognitive Therapy (MBCT)

Another well known approach to mindfulness is Mindfulness Based Cognitive Therapy (MBCT), described by Segal, Williams and Teasdale in 2002[174]. MBCT represents a mixture of training in Buddhist mindfulness meditation, and a cognitive-behavioural conceptual framework aimed specifically at treating and preventing relapse in the context of recurrent major depressive disorder[175]. The hypothesis informing MBCT is that recurrent depression encourages the formation of progressively more stable dysfunctional cognitive patterns, which, in turn, predispose people to develop more frequent relapses[175]. People risk being trapped in an accelerating cycle of depression and relapse. The aim of MBCT is to reduce the likelihood of recurrence by enabling people at risk to become more adept at recognizing dysfunctional mind states, and more skilled at responding appropriately, rather than resorting to rumination or avoidance[175]. Like MBSR, MBCT is also a group-based, 8 week, complex intervention; it includes similar meditation components to those used in MBSR, alongside a more structured approach to the cognitive aspects of depression. MBCT has also been adapted for use in disorders other than recurrent depression, including generalised anxiety disorder[176-178], social anxiety disorder[179], and obsessive compulsive disorder[180].

1.3.3. The evidence base for mindfulness approaches in the treatment of depression:

An evidence-map prepared by the Evidence-based Synthesis Program (ESP) Center at the West Los Angeles VA Medical Center for the U.S. Department of Veterans Affairs[181] concluded that the most consistent evidence for effect of mindfulness-based interventions, including (but not limited to) MBSR and MBCT was in depression. Most published studies utilised either MBCT or MBSR, but there were also positive outcomes with other mindfulness-based interventions for depression. Estimated treatment effect sizes in a range of populations (including recurrent major depressive disorder, those with a history of depression, and depression in the context of other disorders) ranged from Cohen's d 0.23[182]-0.51[183] and Hedges' g 0.23[184]-0.72[185]. The comparator treatments used in the control groups in the studies included in the systematic reviews and meta-analyses consisted mostly of waiting list or treatment as usual controls. One review, which reported two randomised controlled trials comparing MBCT to maintenance treatment with antidepressant medication, concluded that MBCT was at least as effective as maintenance antidepressants for preventing recurrence of Major Depressive Disorder (Risk Ratio 0.80; 95% CI 0.60, 1.08 favouring MBCT, p=0.15)[186]. A meta-analysis of trans-diagnostic psychological treatments for the treatment of anxiety and depression[187] did not find a statistically significant difference in depression outcomes between CBT and mindfulness-based treatments. A meta-analysis of psychological interventions for relapse prevention in depression[188] demonstrated that compared to controls, MBCT reduced the average risk of 12-month recurrence by 21% (RR=0.79,95% CI 0.69; 0.91, I^2 =0%), compared to 25% for CBT and 22% for Inter Personal Therapy (IPT). There was no statistically significant difference between the

modalities at 12-month follow up (chi^2 = 0.21, df = 2 (p = 0.90), I^2 = 0%). A meta-analysis of standardized mindfulness-based interventions in healthcare[189] concluded that MBSR and MBCT were associated with significantly improved depressive symptoms (Cohen's d=0.37; 95%CI 0.28 to 0.45, n=2814). A meta-analysis of RCTs including only participants who were diagnosed with a current episode of depressive or anxiety disorder (N=12 studies; n=578 participants) concluded that there were statistically significant improvement in depressive symptom severity in mindfulness-based interventions (MBIs) vs. control groups (Hedges g =0.73, 95% CI = -0.09 to -1.36), but not in symptoms of anxiety. There were significant effects of MBI vs. inactive controls (Hedges g =-1.03, 95% CI =-0.40 to -1.66), but not vs. active controls. When MBSR and MBCT were separately analysed, significance advantage was found for MBCT vs. control (Hedges' g =-0.39, 95% CI =-0.15 to -0.63) but not for MBSR vs. control (Hedges g =-0.75, 95% CI = 0.31 to -1.81)[190].

1.3.4. The evidence base for mindfulness approaches in the treatment of anxiety:

The evidence-map prepared by the Evidence-based Synthesis Program (ESP) Center at the West Los Angeles VA Medical Center for the U.S. Department of Veterans Affairs[181], examined the effects of a range of mindfulness-based interventions as assessed in published systematic reviews and meta-analyses. The evidence map identified two systematic reviews assessing psychological wellbeing across different clinical populations (excluding healthy subjects). The larger review (36 RCTs) included MBCT and MBSR RCTs and in addition also studies involving other modalities of meditation (Zen, Vipassana, and other types of meditation). This review found statistically

significant positive effects of mindfulness interventions vs. control interventions in anxiety at 8 weeks (Cohen's d=0.38; 95% CI 0.12-0.64) and at 3-6 month follow-up (Cohen's d= 0.22; 95% CI 0.02-0.43)[182]. A systematic review of MBSR for improving health, quality of life, and social functioning in adults[191] which included 31 RCTs (n=1456, mixed population including participants with psychological and/or somatic problems as well as healthy subjects) found a positive effect of MBSR on measures of anxiety (Hedges' g=0.53 [95% CI 0.43, 0.63]). A meta-analysis of mindfulness-based interventions in anxiety and depression[192] which included 39 RCTs demonstrated a statistically significant effect of mindfulness based interventions on measures of anxiety, with an average pre-post Hedges' g of 0.63 (95% CI : 0.53-0.73, p < 0.01). A meta-analysis of MBSR in adults with chronic medical disorders[193], included 4 RCTs, and demonstrated a significant effect on anxiety measures (Hedges' g = 0.47 [95% CI 0.11–0.83]; p < 0.05). A meta-analysis of mindfulness-based interventions for anxiety and depression in patients with cancer[194] included 7 RCTs (n=888); the pooled standardized mean difference (SMD) of the change in anxiety significantly favored mindfulness-based interventions vs. control interventions (-0.75 [95% CI -1.28, -0.22]; p = 0.005). A meta-analysis of trans-diagnostic interventions for anxiety demonstrated a significant effect of mindfulness/acceptance interventions (N=6 RCTs) on anxiety measures (Hedges' g = 0.61 [95% CI 0.37–0.86])[187]. A recent meta-analysis of treatments for anxiety[195] reported that the pre-post effect size for mindfulness interventions in anxiety (N=4 RCTs) was numerically the highest compared with all psychotherapies (Cohen's d = 1.56 [95% CI: 1.20–1.92]). A meta-analysis of MBSR in healthy subjects[196] reported a significant pre-post effect on anxiety measures (8 RCTs) (Hedges' g = 0.55 [95% CI: 0.19 - 0.92] p < 0.005). A meta-analysis of Mindfulness Based Interventions

(MBIs)[197] showed that compared to control interventions, MBIs did not have a statistically significant advantage in reducing anxiety symptom severity (Hedges' g = -0.55, [95% CI = -1.18 - 0.09]). A meta-analysis of MBSR in survivors of breast cancer[198] identified 9 RCTs (n=964; there was a significant advantage for MBSR vs. control group in reducing anxiety (Mean Difference = 2.79 [95 % CI: 1.62–3.96]; p < 0.00001). A recent overview of 5 systematic reviews and meta-analyses[189], (n=2525) demonstrated that compared to waiting list or treatment as usual controls, MBCT and MBSR significantly improved symptoms of anxiety (Cohen's d = 0.49 [95% CI: 0.37-0.61]).

1.3.5. How does mindfulness work?

Despite the growing body of evidence linking mindfulness-based interventions with improvement in depression and anxiety, current understanding of the underlying mechanisms by which these interventions work remains unclear[199]. MBSR and MBCT are complex interventions, each comprised of several components within a facilitated group framework, which also includes intensive personal practice. It is still unclear which of the interventional components are the 'active ingredients' and what the minimum set of interventions necessary and sufficient to bring about positive change in people with anxiety is[200]. There are several potential gains to be made by clarifying the mechanisms by which mindfulness-based interventions bring about improvement in anxiety. First, understanding the mechanisms may facilitate the development of more streamlined approaches, doing away with 'non-essential' components of MBCT/MBSR, potentially shortening the course and simplifying the intervention. Second, clearly identifying the 'active ingredients' may allow the development of more potent mindfulness-based interventions. Third, understanding the mechanisms by which

mindfulness-based interventions work in anxiety may enable transfer of some of these mechanisms in order to enrich/strengthen existing non-mindfulness-based therapeutic approaches, and the development of novel treatments for anxiety.

1.3.5.1. Mechanisms underlying clinical change associated with mindfulness

Baer [201] reviewed the mindfulness literature and suggested several mechanisms that may explain how mindfulness training leads to positive clinical change:

- Exposure: Mindfulness training involves prolonged periods of sitting meditation during which participants are encouraged to engage with whatever comes up in the field of awareness, while maintaining a non-judgmental attitude. Sustained observation of anxiety-related sensations may lead to reduced emotional reactivity, and facilitate habituation and extinction.

- Cognitive change: Mindfulness may lead to changes in the form and content of thoughts, as well as in one's attitude to one's thoughts (e.g. seeing thoughts as 'just thoughts' rather than reflections of true reality).

- Self-management: Mindfulness-related self-observation skills can lead to improved ability to cope with difficult feelings and situations.

- Relaxation: Mindfulness practice may be associated with potentially beneficial relaxation effects (although mindfulness does not have relaxation as a primary aim)

- Acceptance: Acceptance can be defined as *"experiencing events fully and without defense, as they are"*[202]. In the context of mindfulness acceptance is

seen as a prerequisite to change, which is allowed to unfold, rather than forced to occur.

1.3.5.2. Shapiro's theoretical model of mindfulness

Shapiro and colleagues[200] propose a theoretical model of mindfulness based on three axioms that represent an unpacking of the common definition of mindfulness as *"...paying attention in a particular way: on purpose, in the present moment, and non-judgmentally"*[203]:

- *Intention:* Meditators training in mindfulness were found to have a gradually shifting sense of intention; from initial self-regulation, through self-exploration, to self-liberation. They also described their attainments, the results of their growing mindfulness as being in line with their stated intentions[204].

- *Attention:* In a mindfulness context, attention refers to the ability to observe one's internal and external experience with minimal interference. Mindfulness training involves developing attentional skills including concentration (the ability to consistently maintain narrow or wide attentional focus), the ability to monitor attention itself (meta-attention), to shift attention between objects, and to inhibit secondary elaboration.

- *Attitude:* The attitudinal foundations of mindfulness[205] include acceptance, kindness and openness in the face of whatever is present. The concept of orientation to experience includes curiosity, non-striving and acceptance[206].

- These axioms serve as the basis for a proposed theory of mindfulness – the authors propose that *"...intentionally (I) attending (A) with openness and non-judgmentalness (A) leads to a significant shift in perspective, which we have*

termed reperceiving". Reperceiving is seen as a meta-mechanism of action, which overarches four direct mechanisms leading to positive change. These direct mechanisms are: (1) self-regulation, (2) values clarification, (3) cognitive, emotional, and behavioral flexibility, and (4) exposure.

1.3.5.3. Holzel's analysis of mindfulness components

Holzel and colleagues[207] propose several components through which mindfulness exerts its effects:

- *Attention regulation:* The first task most meditators undertake is to stabilize attention by developing concentration – the ability to maintain attentional focus on a particular object. Maintaining this focus despite internal and external distractions requires attentional control. Experienced meditators showed enhanced executive control and orienting performance on the ANT compared to non-meditators[208,209]. A short course of meditation training was associated with improved executive control performance[210], and orienting[208] network performance on the ANT. Experienced meditators showed improved alerting network performance on the ANT following attendance of a meditation retreat[208].

- *Body awareness:* Bodily sensations are a common focus for meditation practice; they are seen as centrally important in the conscious experience of feelings (emotions)[211,212] and for the development of empathy[213]. Meditators report improved ability to attend to bodily sensations[214], but there is little objective evidence in the literature to support this.

- *Emotional regulation*

- o *Reappraisal*: "…the adaptive process through which stressful events are re-constructed as beneficial, meaningful, or benign"[215].

- o *Exposure, extinction, and reconsolidation:* Mindfulness practitioners are asked to attend to whatever comes into the field of awareness, turning towards them with an attitude of acceptance[216]. This has important parallel features with exposure therapy, which leads to extinction of anxiety/fear.

- *Change in perspective on the self*: The Buddhist view of self holds that the perception of a permanent, unchanging, separate self, is the product of an active mental process which is continually producing the appearance of a separate self.[217]. Mindfulness practice leads to the development of meta-awareness, which enables the meditator to become aware of the automatic process of repeatedly constructing oneself – this can lead to progressive dis-identification from a static self. Buddhist psychology sees identification with a solid self as the root cause of suffering – and therefore, the route to the cessation of suffering leads towards seeing the illusory nature of the self. *When it is realized that no self is to be found in the elements of our experience, it begins the process of liberation. Understanding that our sense of "I" is not as solid, permanent, or substantial as we habitually hold it to be ultimately uproots clinging, attachment, and hostility. Understanding this burns up the fuel that runs our repetitive habits. Those who have understood this report a sense of spacious lightness and freedom. They exhibit deep concern and tenderness for others"*[218].

- *Self-compassion:* Self compassion is composed of self-kindness (viewing oneself as a suffering being rather than being harsh to oneself), common humanity (seeing one's experiences as part of a larger context of being human, rather than feeling isolated), and mindfulness ("*holding one's painful thoughts and feelings in balanced awareness rather than over-identifying with them*")[219].

1.3.6. Mindfulness and attention

Mindfulness training is directly concerned with developing attentional skills, beginning with the development of concentration - the ability to maintain prolonged attentional focus on a particular object despite internal and external distractions, and continuing throughout the course of mindfulness training[207]. It is therefore not surprising that effects of mindfulness on measures of attention have been found following both brief and intensive mindfulness-based interventions: experienced meditators showed enhanced executive control and orienting performance on the ANT compared to non-meditators[208,209]. A short course of meditation training was associated with improved executive control performance[210], and orienting[208] network performance on the ANT. Experienced meditators showed improved alerting network performance on the ANT following attendance of a meditation retreat[208]. Brief mindfulness training was associated with improved performance on an N-back task[220]. Intensive mindfulness retreat training (1-3 months) was found to be associated with improved performance on dichotic listening tasks[221,222], with improved detection of target stimuli post distraction, and with reduced distractor attention resource allocation[223]. Improved working memory, sustained attention and performance on a

switching task were associated with novice meditators after attendance at a 10 day meditation retreat[224].

1.4. Trans-cranial direct current stimulation (tDCS)

1.4.1. tDCS Background

tDCS is a non-invasive brain stimulation modality, which changes cortical tissue 'excitability' as a result of applying a weak (0.5-2mA) direct current via scalp electrodes overlying targeted cortical areas. Although there are sporadic mentions of using live torpedo fish to administer electrical stimulation to the scalp of patients with headaches and epilepsy, tDCS originated in the early work of Giovanni Aldini, professor of experimental physics in the University of Bologna in the late 17th and early 18th Century. Aldini, who was Galvani's nephew, developed the first direct current stimulators and used them to treat a wide range of disorders, including melancholia[225-227]. tDCS was used during the 19th and early 20th centuries for treating mental disorders, but was largely abandoned following the advent of electro-convulsive therapy (ECT) in the 1930s[226]. tDCS studies in neuro-psychiatric disorders have been gathering pace since the 1990s, and there is a growing literature on the use of tDCS for a wide range of indications including depression, anxiety, post-stroke neurological rehabilitation[228], Parkinson's disease[229], pain[230], and addictions[231]. In contrast to other neurostimulation modalities, tDCS does not directly trigger action potentials in

neuronal cells, but instead changes overall tissue excitability, and therefore may be more aptly regarded as a 'neuro-modulatory' rather than a neuro-stimulatory approach [232]. Cortical tissue underlying the anode (positive electrode) becomes hypo-polarized, and therefore hyper-excitable; areas underlying the cathode (negative electrode) become less excitable as the average resting potential becomes more polarized. The magnitude of these membrane polarization changes is not in itself sufficient to directly cause neurons to fire [233]. These effects continue after electrical stimulation ceases, and a single application can be associated with tissue excitability changes lasting more than 60 minutes [234,235]. These findings suggest tDCS is likely to be associated not only with transient membrane polarization changes, but also with longer-lasting synaptic changes [236], including anodal stimulation induced long-term potentiation (LTP) and cathodal stimulation associated long-term depression(LTD)[237,238]. The immediate membrane resting potential depolarisation effects of anodal tDCS are abolished by co-administration of carbamazepine, a sodium channel blocker[239]. The longer-lasting, synaptically mediated effects of tDCS are dependent on the glutamatergic system, and are abolished by the N-methyl-d-aspartate (NMDA) receptor antagonist dextromethorphan[239], and significantly strengthened and prolonged by the NMDA agonist D-Cycloserine[240]. Dopamine D_2 blockade using sulpiride abolished the induction of tDCS after effects, whereas the dopaminergic agonist pergolide significantly enhanced these effects[241].

1.4.2. tDCS and neuro-cognitive function:

tDCS has been shown to have effects on several aspects of executive function[242]:

- *Task switching:* A single session of anodal tDCS over DLPFC or primary motor cortex (M1) improved performance on two separate (cognitive and motor) set-switching tasks; whereas cathodal tDCS reduced performance (increased switching costs)[243].

- *Updating:* Anodal tDCS over left DLPFC (1mA, 10min) was associated with improved memory updating on a three back task; neither anodal stimulation of M1, nor cathodal stimulation of left DLPFC were associated with improved memory updating[244]. Anodal tDCS of left DLPFC (30 min,1mA) was associated with improved memory updating on a three-back task vs. sham tDCS[245].

- *Inhibition:* Anodal tDCS over the right inferior frontal gyrus (rIFG) improved response inhibition on the stop-signal task vs. sham stimulation[246].

Anodal tDCS (30 min, 2mA) over the right sphenoid (F10) with extracephalic cathode, was shown to enhance the alerting (but not the orienting or executive) attention network on the ANT vs. sham tDCS [247]. Anodal tDCS over Frontal eye fields (FEF) (1mA, 10min) was associated with shortening of pro-saccade latency, and with reduced anti-saccade error rate; cathodal tDCS was associated with anti-saccade latency prolongation[248]. Anodal tDCS over left DLPFC with cathode over left temporoparietal junction (2mA, 30min, twice daily, for 5 days) was associated with reduction in anti-saccade error percentage ($p = 0.005$) and with reduced severity of auditory hallucinations ($p = 0.003$) in patients with schizophrenia[249].

tDCS of the DLPFC has been shown to modulate a wide range of cognitive abilities including working memory, executive function, risk taking, emotional processing,

verbal learning, problem solving, and decision making (for a review see Tremblay et al. 2014[250]). A recent quantitative review of single session tDCS studies in healthy subjects[251] concluded that tDCS was found to not have a significant effect on executive function, language, or memory; critics of this review's findings pointed out *"substantial problems with their data selection and statistical approach severely undermine confidence in their findings"* [252].

1.5. Summary and conclusions

Anxiety is a part of the normal spectrum of human experience. Anxiety disorders are an important cause of worldwide morbidity, due to their prevalence, impact on quality of life, and associated economic, and societal burden. GAD is an important anxiety disorder, whose core feature is worry. Existing treatments for anxiety disorders have significant shortcomings; therefore, new treatments for anxiety disorders (particularly for GAD) are needed.

Theoretical models and experimental findings point towards threat-related cognitive and emotional processing biases in anxiety. The attentional control theory (ACT) states that anxiety impairs attentional control through effects on central executive functions (inhibition, task-shifting, and updating). These effects shift the balance of attention resource allocation from top-down (goal-oriented) towards bottom-up (stimulus-driven) systems. Anxiety-related processing biases necessitate the allocation of additional cognitive resources (thus reducing performance efficiency) in order to maintain performance effectiveness. These effects are particularly prominent in the presence of threat-related stimuli, but can also be detected in the absence of perceived threat.

Attention is a complex phenomenon underpinned by three inter-related, anatomically and functionally distinct networks: The alerting, orienting, and executive attentional networks. State and trait anxiety have distinct patterns of modulating these networks.

The anti-saccade task is used for investigating subjects' executive function. The task requires the suppression of a reflexive action, replacing it with a volitional task. It enables measurement of performance efficiency and effectiveness.

The Attention Networks Task (ANT) task is a simple computerized test used for measuring performance of the three Attentional networks.

Inhalation of 7.5% CO_2 is a reliable method for inducing anxiety in healthy volunteers and in patients with GAD; it is gaining acceptance as a validated model for GAD.

Mindfulness-based interventions have a substantial evidence base for efficacy in depression and a growing evidence base in anxiety disorders, with a good profile of acceptability and safety in both clinical and pre-clinical populations. Mindfulness has been shown to have effects on executive function and attention.

Transcranial direct current stimulation (tDCS) is a novel neuro-modulatory intervention with a growing evidence base in a range of disorders and conditions. tDCS has been shown to have effects on executive function and on attention.

Summary of research aims and overview of thesis:

This thesis presents a sequence of chapters, starting with chapter 2: a systematic review and meta-analysis of tDCS for the treatment of depression, and a review of tDCS for the treatment of anxiety disorders. The thesis goes on to describe a series of studies exploring the effects of mindfulness-based interventions and of a single session of tDCS in healthy volunteers using the anti-saccade task and the ANT to assess the effects of

these interventions on measures of attention and on cognitive processing biases related to 7.5% CO_2 inhalation as a model for GAD:

- Chapter 3: Investigation of the effect of meditation training on attention network functionality (measured using the ANT) in healthy volunteers. The study compared two types of meditation training (open monitoring and focused attention) and a test-retest control group.

- Chapter 4: Investigation of the effect of mindfulness training on attention control (measured using the anti-saccade task) in healthy volunteers.

- Chapter 5: Investigation of the effect of mindfulness training on attention control (using the anti-saccade task) in healthy volunteers under conditions of experimentally induced anxiety, utilising the 7.5% CO_2 challenge. The study compared two types of meditation training (open monitoring and focused attention) and a relaxation control group.

- Chapter 6: Investigation of the effects of a single session of active vs. sham frontal tDCS on attention network functionality (measured using the ANT) in healthy volunteers.

- Chapter 7: Investigation of the effects of a single session of active vs. sham frontal tDCS on attention control (using the anti-saccade task) in healthy volunteers under conditions of experimentally induced anxiety, utilising the 7.5% CO_2 challenge.

- Chapter 8 concludes the thesis with a general overview and discussion.

2. CHAPTER 2: THE EVIDENCE BASE FOR TDCS IN DEPRESSION AND IN ANXIETY DISORDERS

2.1. Introduction

Chapter 1 of this thesis described the importance of anxiety as a cause of morbidity on a global scale, and made the point that existing treatments for anxiety disorders have significant limitations in their efficacy, and acceptability. It therefore clear that exploring the utility of new treatments for anxiety disorders would be an important contribution to the health of people across the globe. tDCS has been suggested as a potentially useful treatment for anxiety disorders, but the evidence-base for its use in these disorders is still in its infancy. It is important to have a full understanding of the existing evidence, before progressing to studies of tDCS as a potential treatment for anxiety disorders in Humans. This chapter will address the evidence base for tDCS in depression and in anxiety. It includes a systematic review and meta-analysis of tDCS in depression, and a review of the literature describing the use of tDCS in anxiety disorders. The meta-analytic approach was necessary in order to combine all of the published RCTs of tDCS in depression, with a view to addressing current controversies based on inconsistent findings in the literature. The evidence-base for tDCS in anxiety disorders is not yet sufficiently developed to enable a meta-analysis to be undertaken; we therefore conducted a systematic review of the existing evidence.

2.2. Transcranial direct current stimulation (tDCS) in the treatment of depression: systematic review and meta-analysis of efficacy and tolerability[253]

2.2.1. Introduction

Depressive disorders are prevalent, recurrent, often run a chronic course, and are associated with significant worldwide morbidity and mortality[254,255]. Treatment with antidepressant medication is often suboptimal in terms of efficacy, safety and tolerability[256,257]. Psychological interventions are associated with significant rates of suboptimal effectiveness, even when combined with antidepressant medication[258]. Electro-Convulsive Therapy (ECT) is highly effective, but is associated with significant stigma, and adverse effects[258]. Other invasive and non-invasive neurostimulation modalities have been proposed for the treatment of depression, but their utility may be limited by issues such as cost, tolerability and availability[232]. In particular, there are important differences between tDCS and repetitive transcranial magnetic stimulation (rTMS) in terms of adverse effect profiles, focality of stimulation, and also in the cost, availability and portability of equipment[7]. Trans-cranial direct current stimulation (tDCS) is a novel treatment modality for depression, which may represent an alternative to pharmacological or psychological treatments. The body of research describing the efficacy, safety and tolerability of tDCS in depression is growing. Three earlier meta-analyses have been published [259-261], these used different methodologies and produced inconsistent findings. We therefore performed a systematic review and meta-analysis of the efficacy and tolerability of tDCS in depression, using a comprehensive set of meta-analytic tools, and incorporating all published randomised controlled trials to date.

2.2.2. Method

A literature search and meta analysis were conducted following the recommendations of the Cochrane collaboration[262] and the *PRISMA* guidelines[263]. Two authors (DM and NH) performed the systematic review and data extraction. All discrepancies were resolved by consensus.

2.2.2.1. Literature Review

We searched the PubMed database using the following search strategy:

((((((("direct"[Title/Abstract]) AND "stimulation"[Title/Abstract])) OR "tdcs"[Title/Abstract])) AND (("rand*"[Title/Abstract]) OR "control*")) AND "depress*"[Title/Abstract]. The date range extended up to April 30th, 2015. We also scrutinized the reference lists in published meta-analyses of tDCS in depression and articles listed as citing these meta-analyses[259-261].

Inclusion criteria used were: English language publications; Randomised, sham-controlled trials; including data enabling calculation of effect size for depression rating scale change, and/or response/remission rates; patient population with depressive disorders; tDCS as monotherapy or augmentation therapy for treatment of depression.

Exclusion criteria used were: Studies in animals; non-controlled or non-randomised trials; case reports / case series; trials of treatments for disorders other than depression; trials of interventions other than tDCS; duplicated data sets.

2.2.2.2. Data Extraction

The following data were extracted: Population demographics including sample size; diagnosis (unipolar/bipolar depression); tDCS characteristics (including number of sessions, montage, current used, inter-session intervals, sham stimulation

characteristics); efficacy outcome measures and outcomes (Including rating scale score changes and response/remission rates); acceptability (using dropout numbers as a proxy measure).

Outcome measures included both continuous depression rating scale scores, and categorical response/remission rates - we included both types of outcome for the following reasons: First, previous meta-analyses utilised continuous outcome measures [259], categorical outcome measures [260], or both [261], which may explain the inconclusive and at times contradictory nature of their findings. Second, whereas continuous outcome measures may offer superior sensitivity, their specificity is considered inferior to that of categorical outcome measures. Third, while categorical response/remission rates may be more clinically 'meaningful', they require larger sample sizes, and as all studies included in our meta-analysis utilised continuous primary outcome measures, they may have lacked sufficient statistical power to reliably test hypotheses based on categorical outcomes.

We therefore extracted the following data: for continuous outcomes we meta-analysed depression rating scale scores at randomised blinded treatment endpoint, using the study primary outcome measure rating scale; for categorical outcomes, we meta-analysed remission and response rates for active and sham groups at randomised blinded treatment endpoint. Response was defined as ≥ 50% reduction in depression rating scale score from baseline to endpoint; we used the specified remission criteria provided by each study.

2.2.2.3.   Meta Analysis

Our adopted meta-analytic approach makes several important contributions to the literature (Table 2.2). We:

1. Increase the number of included RCTs and subjects, compared to previous meta-analyses.

2. Use a methodology combining continuous outcome measures (rating scale scores) with possibly more clinically relevant dichotomous measures (i.e. response and remission rates).

3. Perform moderator analyses to clarify the effect of putative moderators identified in narrative analysis.

4. Perform power and precision analyses to inform future research in terms of sample size planning.

5. Identify important gaps in knowledge and suggest new directions for future research, methodological improvements and improved reporting standards.

6. Examine the use of tDCS in conjunction with antidepressant medication and with Cognitive Control Training (CCT)

7. Clarify in which conditions tDCS might be clinically useful.

The primary effect size index used to quantify the continuous treatment effect was Hedge's g - the difference in the reduction in depression severity rating scale scores (MADRS and/or HDRS) between the two groups (active tDCS – sham tDCS). This was calculated by subtracting the Hedge's g for the difference in depression ratings between groups before treatment from the corresponding difference immediately after treatment. A positive value of g represents a larger decrease in depression

severity rating in the treatment group, relative to the control group. Our choice of standardizer for *g* was the pooled within-groups *SD (SDpooled)*, as this has more degrees of freedom (since it is derived from two groups) than other standardisers and is thus likely to be the most precise estimate of the population *SD*. We chose the "bias corrected" Hedges *g* since it provides superior point estimates over Cohen's *d* which inflates point estimates for small samples. However, following best practice, our confidence intervals were uncorrected, since simulations show that confidence intervals on *d* provide more accurate interval estimates[264]. For each study, we computed *g* and 95% confidence intervals by using a combination of means, standard deviations, independent samples *t*, *p* and *F* statistics using the R programming language. In practice, these were the same routines implemented by the widely used Comprehensive Meta Analysis Software (CMA: Biostat, Englewood, NJ).

We performed two additional meta-analyses to quantify the overall treatment effect in terms of categorical response and remission rates[256]. In both cases, we compared the differences between treatment and control groups by using the log odds ratio as an effect size index. A positive score represents a higher likelihood of response or remission in the treatment (active tDCS) group, relative to the control (sham tDCS) group. Response was defined as 50% reduction in depression rating scale score from baseline to endpoint. We used the remission criteria provided by each study.

As previous meta-analyses have indicated considerable heterogeneity in effect size estimates between studies [259], we made an *a priori* decision to analyse our effect size data using a random effects model, due to its tolerance of heterogeneous effect sizes

and conservative nature of estimation [264,265]. The random effects model assumes that each study estimates different values from a distribution of population parameters, rather than assuming studies are direct replications of each other. We assessed heterogeneity across effect sizes by using Cochran's Q and I^2 statistics. Unless reported otherwise, parameter estimates were obtained via restricted maximum likelihood estimation, due to its accuracy relative to other estimators with smaller numbers of studies[266]. Statistical tests of model coefficients were computed via Wald-type chi squared tests. We additionally used a pseudo-R^2 statistic to assess the amount of heterogeneity between effects explained by including moderators [267]. R^2 estimates the proportional reduction in heterogeneity after including moderators. For interpretation, it is important to note that this pseudo-R^2 does not include sampling variability, meaning that it is possible to get very large R^2 values, even when there are discrepancies between the model and the observed effects (provided these are not larger than expected by sampling variability). Model comparisons were conducted via likelihood ratio tests. All meta analyses were performed using the "metafor" package in R[268].

To account for heterogeneity across treatment effects, we assessed the impact of potential categorical and continuous moderators of the treatment effect. The moderators we examined are listed in table 2.1. Moderator data for each study were recorded in a structured fashion. Where the information was reported, we recorded both categorical moderators and continuous moderators. Two experienced authors (NH, DM) acted together as coders, and no disagreements on coding decisions were encountered.

Potential moderator	Units	Descriptive Statistics
Current	mA (Boolean)	*k* = 11 1mA (*k* = 2) 2mA (*k* = 9)
Course delivered	Number of sessions	*k*= 11 *M*= 9.55 *SD* = 3.50 Range = 5-15
Session duration	Minutes per session (Boolean)	*k* = 11 20 mins (*k*=7) 30 mins (*k*=4)
Total tDCS time	Number of sessions x session duration (minutes)	*k*= 11 *M* =229.09 *SD* = 87.80 Range = 100- 300
Total current delivered	mA x number of sessions x session duration	*k* = 11 *M* = 430.91 *SD* = 210.97 Range = 100- 600
Inter-session intervals	Days (Boolean)	*k* = 11 1 day (*k*=9) 2 days (*k*=2)
Diagnosis		*k=11* unipolar (*k*=9) unipolar and bipolar (*k*=2)
Concurrent antidepressant medications (ADMs)		*k=11* Concurrent ADMs *(k=8)* No-concurrent ADMs *(k=3)*
Concurrent cognitive control therapy (CCT)		*k=11* Concurrent CCT *(k=2)* No concurrent CCT *(k=9)*
Treatment resistance level	Number of previous courses of antidepressants failed in current episode	k= 9 (see table 2.3)

Table 2.1: variables examined as potential moderators of tDCS outcomes in depression.

These include both participant-related and tDCS-related factors, with a view to enable meta-analytical examination of the variables found to be associated with tDCS outcomes in the narrative analysis.

2.2.2.3.1.   Additional analyses: publication bias, precision and power

To assess the impact of the so-called "file drawer problem", whereby unpublished null results can distort meta-analytic estimates, we performed 'fail-safe N' analyses using the methods developed by Orwin [269] and Rosenthal [270]. This allowed estimation of the total number of unpublished studies averaging null effects that would be required to reduce the reported treatment effect size to target levels [269] or to statistical non-significance [270]. Despite the existence of other meta-analyses estimating the magnitude of tDCS efficacy, to date there has been no formal, explicit attempt to use these parameter estimates to inform future research planning. We hence addressed this issue from both an Accuracy In Parameter Estimation (AIPE), and power analytic perspective. The AIPE analyses were used to estimate the appropriate sample size for future trials such that the expected width of the confidence interval meets a desired level of precision [264]. Unlike power analysis, this approach negates the need to invoke a null hypothesis significance test, and instead is solely concerned with precision in parameter estimation. This was achieved by using the non-central t method described by Kelley[271], implemented in the "MBESS" package in R [272]. To provide an assessment of the statistical power of individual studies, we estimated the power of each study to detect the summary effect estimated in the meta-analysis at a nominal level of significance (p=.05). By extension, for future sample size planning, we calculated the minimum N required to achieve adequate (80%) power to detect the estimated summary effect.

2.2.3. Results:

2.2.3.1. Randomized Controlled Trials (RCTs):

We identified ten randomized controlled trials of tDCS in depression using PRISMA methodology (Figure 2.1a), and quality-assessed the each trial using the Cochrane Collaboration's tool for assessing risk of bias in randomised trials[273] – this yielded an acceptable profile of Risk of Bias across the RCTs included in the meta-analysis (Figure 2.1b). Due to the diversity of study designs, we made the following decisions about the analysis of individual studies: 1. Boggio et al. (2008) [274] randomised participants to 3 groups: active tDCS over left DLPFC, sham tDCS over left DLPFC, and active occipital tDCS; we chose to exclude the occipital group from our meta-analysis. 2. In analysing the Brunoni et al. 2013 trial[275] , we separated the data from participants who received sertraline and those who received placebo medication - this created two separate effects (hence although we included 10 RCTs, we analysed k=11 effects).3. Segrave et al (2014)[276] randomised participants to 3 groups: 1.tDCS + Cognitive Control Training (CCT), or 2.sham tDCS + CCT, or 3. tDCS + sham CCT - For the purpose of this meta-analysis, we included only 2 of the 3 study arms – comparing tDCS+CCT vs. sham tDCS+CCT. 4. Several studies reported primary outcomes at endpoints, which included a follow-up period after the end of active treatment course. For the sake of consistency, we analysed all available data at the point when active blinded treatment ceased.

2.2.3.1.1.   Fregni 2006[277].

Study. A randomised double blind sham controlled trial in which 18 outpatients with (unipolar) MDD who had not been prescribed antidepressant medication for at least three months prior to inclusion were randomised to 2 treatment arms: either active tDCS, in which the anodal electrode was placed over F3 (10-20 International EEG System), and the cathode over the right supra-orbital area, a current of 1mA being applied for 20 minutes on 5 alternate days; or sham tDCS with similar settings, but the stimulator being switched off after 5 seconds. Outcome measures involved a battery of neuropsychological rating scales; mood was assessed with the HDRS. The active treatment group showed a significantly greater improvement in mood, mean Hamilton Depression Rating Scale (HDRS) scores reduced by 58.5% (+/-20.4%) vs. 13.1% (+/-23.4%) in the sham tDCS group [$F(1,16)=19.2$, $p<0.001$]. tDCS was generally well tolerated, and no complications were reported. The active tDCS group improved on all cognitive tests, compared to their baseline scores. There was no significant cognitive improvement in the sham tDCS group. Improvement in cognitive function in the treatment group was not correlated with the improvement in mood, suggesting that cognitive and affective changes were mediated by different mechanisms.

Comment. Although involving a small number of participants, this study found evidence for antidepressant efficacy and tolerability of tDCS in MDD. The sample baseline characteristics were well defined, with moderate-to-marked baseline depressive severity (mean HDRS scores of 23.56+/-5.03 in the active tDCS group and 25.89+/-4.26 in the sham group), significant number of patients had recurrent or persistent illness.

2.2.3.1.2. Boggio 2008 [274].

<u>Study</u>. Following this pilot study, the same group conducted a parallel-group, double-blind, initial clinical trial of tDCS in patients with unipolar depression who had not been prescribed antidepressants for at least two months prior to trial entry. Exclusion criteria included neurological disorders, comorbid Axis I disorders, substance abuse within three months of study participation, psychotic features, bipolar disorder and Axis II disorders. Forty patients were randomized into three treatment arms (using a 2:1:1 randomization strategy): active treatment, with anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) - anode over F3 on the 10-20 International EEG System, cathode over right supraorbital region (N=21); active control, with anodal tDCS of the occipital cortex - anode 2cm above Inion on the midline, cathode over right supraorbital region (N=9); or sham control with sham tDCS over left DLPFC, and cathode over the right supraorbital region, the stimulator being active for an initial 30 seconds only, with ramp-up and ramp-down to mask shamming (N=10). Patients received 10 sessions of tDCS on consecutive working days (no treatment being given at weekends), the current was set at 2mA for 20 minutes in each session. The primary outcome measure was the 21-item HDRS, the BDI being the secondary outcome measure. Patients were rated at baseline, at end of treatment and at 15 and 30 days after treatment. tDCS was well tolerated, reported adverse effects being mild (headache, itching or redness at electrode site) and not significantly associated with group assignment. At the end of treatment, there was a significant difference between the active treatment group and the active control group (p=0.009) and the sham control group (p=0.0018) in HDRS score; but the control groups did not separate from each other (p=0.6). The active group maintained separation from the sham control at

the 30-day follow-up (*p*=0.04). BDI outcomes showed a similar pattern, with the active group separating from the sham group at the end of treatment (*p*=0.0045, effect size (Cohen's *d*) =1.11), and at 30-day follow-up (*p*=0.03). There were 8 responders (HDRS scores reduced by at least 50% from baseline) in the active treatment group, compared with 2 in the active control and none in the sham control groups (*p*=0.019). There were 5 patients in remission (HDRS < 8) in the active treatment group, but none in the other two groups (*p*=0.02).

Comment. This study was larger than previous studies of tDCS in depression and demonstrated both acute efficacy and an effect lasting 30 days after treatment was stopped. The findings provide support for the use of left DLPFC anodal stimulation. This was the first study to indicate that the therapeutic effect in depression is related to the anodal effect at left DLPFC rather than to the cathodal effect at the right supra-orbital area. The tolerability of active tDCS was not significantly different to sham stimulation.

2.2.3.1.3.   Loo (2010) [278]

This double-blind, sham-controlled trial of left prefrontal tDCS in depression involved 40 outpatients with DSM-IV MDD with baseline MADRS (Montgomery-Åsberg Depression Rating Scale) score of 20 or more. Exclusion criteria included diagnosis of bipolar disorder, drug or alcohol dependence or abuse, other Axis-I disorders, and neurological disorders; or the failure to respond to ECT during the index episode. Subjects were either medication-free, or had continued on the antidepressant drug to which they had not previously responded at a stable dose, unaltered for at least 4

weeks prior to study enrolment (some patients were prescribed antipsychotic drugs, and 1 patient was prescribed lamotrigine: none were prescribed benzodiazepines). Subjects were randomised to: [i] active tDCS with anodal tDCS over left DLPFC (pF3 on the10/20 EEG International System), the cathode being placed over the right lateral orbital area: 1 mA of current was used for 20 minutes, with 30 seconds of ramping-up at onset; [ii] sham tDCS with a similar montage, the current being ramped down over 30 seconds immediately after initial ramping up. Subjects in both arms underwent treatment three times weekly for five treatment sessions. All subjects then received active tDCS for another five sessions (at the same frequency). After 10 sessions, the blind was broken and patients who had received sham tDCS in sessions 1-5 were then offered another 5 sessions of active tDCS - bringing the total number of active tDCS sessions offered to all participants (regardless of treatment arm assignation) to 10. The primary mood outcome measure was the MADRS, and secondary outcome measures included the 17-item HDRS and Clinical Global Impression of Severity (CGI-S), the patient-rated BDI and Patient Global Impression Scale of Improvement (PGI-I). Subjects were assessed at baseline, after sessions 5, 10 and 15, and at 1-week and 1-month follow-up. Over the sham-controlled phase (sessions 1-5) there were no significant between-group differences in mood outcomes (p=0.87 for MADRS). There were statistically significant differences on all measures comparing baseline mood to mood after session 10, but no significant differences between groups. There were 6 responders (MADRS score reductions of at least 50%) and 5 remitters (MADRS<11) in the active tDCS group, compared to 4 responders and 3 remitters in the sham group. One patient in the sham tDCS group committed suicide on the day following his first active tDCS session: the authors felt this was unlikely to have been related to the

treatment this patient received (the patient had been noted to have suicidal thoughts for some months prior to his death, the suicide occurred on the first instance when he was briefly left on his own, and there were no emotional or clinical changes noted following the session of active tDCS that he had received). Adverse effects reported by the active tDCS group included redness, itchiness and tingling at electrode (mainly anode) sites; mild headaches, light-headedness and ringing in the ears; visual changes including blurring, brighter/illuminated vision; and mild euphoria, transient hypomania (*N*=1), nausea, insomnia and anxiety. After session 10, subjects were asked to indicate whether they thought they had received active or sham tDCS, there being no significant in the accuracy of identification between the two groups.

Comment. This study used similar stimulation parameters to those used by Fregni et al 2006[277,279] (five 20 minute sessions at 1mA on alternate days with similar electrode montage,) but did not show a statistically significant separation between active and sham tDCS groups. The efficacy of active treatment over 10 sessions was comparable to that demonstrated by Boggio et al.[274] who used tDCS at higher intensity (2mA, 5 times weekly for 10 sessions). The main difference between this study and the earlier trials was the greater degree of improvement in the sham tDCS group. Factors which may have contributed to this difference included the participation of patients taking antidepressants and patients with co-morbid Axis II (personality) disorders. The degree of treatment resistance in this cohort was only 'moderate' (mainly stage 0-III on the Thase & Rush system [280], which may explain the magnitude of sham tDCS effects in this trial. The authors acknowledge that main weaknesses in this study, namely its short duration and the small number of treatments in the sham-controlled phase: they state

that a longer sham-controlled phase would have been necessary to demonstrate a difference between active and sham tDCS. They suggest that future studies utilise tDCS at higher intensities (>1mA), with daily treatment sessions, over longer periods, in more treatment-resistant patients.

2.2.3.1.4. Palm (2011) [281]

This randomized double-blind placebo-controlled crossover trial of tDCS in treatment resistant depression included 22 outpatients (14 women, 8 men: mean age 57 years): 20 with unipolar depression (17 with recurrent depression, and 3 with first-episode depression), and 2 patients with bipolar depression. All participants had failed to respond to at least two trials of antidepressants from different classes. Antidepressant medication was kept unchanged for at least 3 weeks before starting tDCS, and no medication changes were made during the study. Patients were randomized to two arms: [i] 10 sessions of active tDCS followed by 10 sessions of sham tDCS; [ii] 10 sessions of sham tDCS followed by 10 sessions of active tDCS. The anode was placed over left DLPFC (F3 on the 10-20 EEG international system), and cathode over right supraorbital region. The first 10 patients received stimulation sessions of 20 minutes at 1mA; the current setting was increased to 2mA for the subsequent 12 patients. All patients received 20 tDCS treatments over 4 weeks. The sham stimulation included 15-second ramp-up and ramp-down periods to simulate active tDCS sensations. Electrodes were soaked in tap water for the initial 15 participants, but this was changed to normal saline solution, due to skin lesions at stimulation sites. The primary outcome measure was the HAMD-24 at 2 weeks and 4 weeks, secondary outcome measures included the BDI, CGI, PANAS and a series of cognitive

tests. Twenty patients completed the trial: no significant differences were found between active and sham tDCS on the primary outcome measure - although modelling the HAMD course via mixed model analysis showed that active tDCS was superior to sham tDCS in weeks 1 and 2 (p=0.0492). Subjective mood ratings showed a significant advantage for active over sham tDCS in positive emotions on the PANAS-pos and a trend for reduced negative emotions on the PANAS-neg. There were no significant between group differences in cognitive measures. Six of the 15 patients undergoing treatment involving use of tap-water soaked electrodes developed crusty skin lesions at the cathode site, but after normal saline solution was substituted, no further lesions were reported. Other adverse effects reported were minor, including slight headache and skin itchiness during treatment. Blinding integrity was not significantly different between groups.

Comment. The failure of active tDCS to separate from sham tDCS in this study may have been influenced by the small sample size, the change in treatment (from 1mA to 2mA) during the study, the cross-over design without an intervening no-treatment period, the higher degree of treatment resistance (average failed antidepressant trials 2.9 vs. 1.0-2.6 in previous trials), the older age of patients (56 years, compared to 46-54 years in previous studies), and the use of concomitant antidepressant medication.

2.2.3.1.5. Loo (2012) [282]

This randomised sham-controlled trial included 64 outpatients with DSM-IV defined major depressive episode (MDE) in the context of both unipolar and bipolar depression, with baseline MADRS score of 20 or more. Exclusion criteria included the

presence of other Axis I mental disorders, excessive alcohol/drug use, neurological disorders, metal implants, history of heart or neurological disease, failure to respond to ECT in the index episode, pregnancy, and treatment with medications known to modulate tDCS effects (including benzodiazepines, anticonvulsants, dextromethorphan and pseudoephedrine). Subjects were either antidepressant medication-free for the duration of the trial, or continued on the antidepressants to which they had previously failed to respond (with no dose changes for at least 4 weeks before starting tDCS). Subjects were randomized to receive either active tDCS at 2mA for 20 minutes, with ramp-up and ramp-down for 30 seconds, or sham tDCS at 1mA for 30 seconds, with ramp-up and ramp-down for 10 seconds. The anode was placed over left DLPFC (pF3 on the 10-20 EEG international system), the cathode lateral to the right orbit (F8 on the 10-20 EEG international system). Treatments were carried out five days per week for three weeks; each subject subsequently being offered another 15 sessions of open-label active tDCS. Treatment responders (i.e. those whose MADRS scores reduced by at least 50% from baseline) were offered further weekly sessions of tDCS during the 1-month follow-up period. The primary outcome measure was the MADRS, assessed at baseline, after sessions 8, 15, 23 and 30, and at weeks 1 and 4 after trial completion. Other measures included the IDS, CGI-S, QIDS-C, QIDS-SR, CORE and a set of neuro-cognitive assessment tools. A significant interaction between group and time was seen on the primary outcome measure (MADRS scores), active tDCS being associated with lower MADRS scores during the sham-controlled phase (p=0.04; effect size 0.49), but there was no significant separation on other mood outcome measures. At 1-week follow-up, 16 out of 26 subjects in the active tDCS group met criteria for response (reduction in MADRS of at least 50% from baseline) compared to 6 out of 26 in the

sham tDCS group. The NNT for response at 3 weeks of active vs. Sham tDCS was 16.7; the NNT for response at 6 weeks of active tDCS vs. 3 weeks of sham tDCS was 2.6. There was one reported case of transient hypomania in the open phase of the trial (the patient had bipolar depression), other adverse effects were transient and mild to moderate in intensity, including skin redness, burning sensation, tingling and itchiness at electrode sites, headache, dizziness and nausea. A blinding integrity test found no significant differences between groups.

Comment. This trial included more participants and employed more robust treatment parameters (in terms of number of sessions, duration of blinded treatment, current settings, and follow-up period) than previous tDCS trials. Using the *a priori* primary outcome measure, this study confirmed that active tDCS has significantly greater antidepressant effects than sham tDCS over the 3-week sham-controlled phase. The magnitude of antidepressant effects (28% reduction in MADRS) was lower than in some previous studies [274,279] but patients in those trials were medication-free and the scope for tDCS-related improvement may have been greater in the absence of medication: a meta-analysis of treatment with rTMS with and without concomitant medication showed a similar finding[283]. The number of responders after six weeks of treatment was superior to the responder rate reported in the STAR*D trial (28.5%)[256].The authors suggest that extending the treatment duration to 6 weeks may be associated with additional gains (though it is unclear whether the benefit is associated with a higher number of treatments, or with the extended duration of treatment). The study findings suggest a broadly equivalent effect size to that observed with antidepressant medication, despite selection of patients with a moderate degree of treatment

resistance, many of whom were already taking antidepressant medication. The results suggest that more robust tDCS treatment parameters, in terms of current, number of sessions and overall duration of treatments, may be associated with better outcomes in depression.

2.2.3.1.6. Blumberger (2012) [284]

This study explored the potential utility of tDCS in treatment-resistant depression. A total of 24 outpatients with a diagnosis of DSM-IV defined major depressive episode (MDE), a baseline HDRS score greater than 20, and fulfilling Stage II criteria (or above) on the Thase Scale for treatment resistance (i.e. failure to achieve remission or tolerate at least two trials of antidepressants from different classes) were recruited. Adjuvant medication (including antidepressants, antipsychotics and benzodiazepines) was permitted, providing dosage was stable for at least 4 weeks before study treatment started and throughout the duration of the trial. Exclusion criteria included treatment with anticonvulsants, DSM-IV substance use disorder in the 6 months prior to potential trial commencement, an unstable co-morbid medical condition, a history of seizures, pregnancy, and DSM-IV borderline or antisocial personality disorder. Subjects were randomised to receive active (N=13) or sham (N=11) tDCS. Treating clinicians were aware of treatment allocation, but patients and outcome assessors were blind to allocation. Fifteen treatment sessions were delivered on consecutive working days, over 3 weeks. The anode was placed over the left DLPFC (F3 according to the 10-20 EEG system), the cathode over the right DLPFC (F4 according to the 10-20 EEG system). Active treatment was delivered at 2mA for 20 minutes, the sham treatment included an initial 30-second period of stimulation at 2mA, and the current was then turned off.

The primary outcome measure was change in HDRS from baseline to endpoint, secondary outcome measures including the proportion achieving symptom remission (HDRS<8) or response to treatment (50% reduction in severity). There was no significant difference between active and sham tDCS in the change in HDRS score (p=0.80): none of the subjects met HDRS remission criteria, and only 1 subject in each group met response criteria; and there were no significant between-group differences in MADRS change or in BDI-II change. No serious adverse events were reported during the trial, though four subjects in each group reported skin tingling. Headache was reported by three subjects in the active group and by none of the sham group subjects: 1 participant in the sham group withdrew due to scalp irritation. The majority (73.7%) of subjects correctly guessed their treatment allocation at trial endpoint (60% in active group and 88.9% in the sham group).

Comment. This sample had a higher degree of antidepressant treatment resistance when compared to participants in previous tDCS trials (the mean number of failed antidepressant trials was larger than 4, 46.2% of the active group and 18.2% of the sham group had a history of treatment with ECT; 23% of the active group and 9.1% of the sham group had failed a trial of ECT in the current episode of depression). This was the first study to focus on use of tDCS in treatment resistant unipolar depression in patients taking a wide variety of concomitant medication. The study limitations include small sample size, and probable under-powering (the power calculation required 48 subjects, but only 24 were recruited): recruitment was stopped on ethical grounds after an interim analysis found no difference between treatment groups. The degree of treatment resistance in this cohort may have been too great to permit a detectable

effect; and blinding may have been sub-optimal. Subjects who started antidepressants four weeks prior to the trial may have experienced antidepressant drug-related treatment effects during the trial. The active stimulation group was more treatment-resistant and more were taking benzodiazepines (which may impair the effects of neurostimulation).

2.2.3.1.7. Brunoni (2013) [275]

This study explored the comparative safety and efficacy of tDCS, the antidepressant sertraline, and placebo, as well as their combinations in the treatment of Major Depressive Disorder. A total of 120 subjects with DSM-IV defined major depressive disorder (MDD), a baseline $HDRS_{17}$ score greater than 17, and a low suicide risk, took part in this double-blind randomized controlled trial. All subjects were free of antidepressant, antipsychotic and anticonvulsant medication for at least 5 half-lives of the drug, before onset of trial. Adjuvant benzodiazepines were permitted. Exclusion criteria included: other axis-I disorders (co-morbid anxiety disorders were permitted), substance use disorders, axis-II disorders, previous neurological conditions, severe axis-III disorders and specific contra-indications to tDCS (e.g. metallic implants in the head). Subjects who were prescribed sertraline in the current depressive episode were excluded. Subjects were randomized to one of four groups: Active tDCS + sertraline, tDCS + placebo medication, sham tDCS + sertraline, sham tDCS + placebo medication. Participants and assessors were blinded to treatment allocation; the treating clinicians were aware of allocation, but their interaction with participants was kept to a minimum. Medication and tDCS were initiated concurrently. tDCS was delivered using a bi-frontal montage, with the anode placed over the Left DLPFC (F3 according to the 10-

20 EEG system), and the cathode over Right DLPFC (F4 according to the 10-20 EEG system). Twelve tDCS sessions were delivered – 10 sessions on consecutive week days (Monday to Friday) and two subsequent sessions at fortnightly intervals. Active tDCS was delivered using a current setting of 2mA, for 30 minutes per session; sham tDCS was delivered by switching the stimulator off after 1minute. Sertraline was administered at a fixed dose of 50mg per day. The primary outcome measure was change in MADRS score at 6 weeks; secondary outcomes were clinical response (defined as >50% reduction of the baseline MADRS score), clinical remission (defined as a MADRS score ≤10), and scores on the HDRS17, Global Impression–Severity of Illness scale, and Beck Depression Inventory). At the main end point (6 weeks), there was a significant difference in MADRS scores when comparing the combined treatment group (sertraline/active tDCS) vs. sertraline + sham tDCS (mean difference, 8.5 points; 95% CI, 2.96 to 14.03; p=.002), tDCS + placebo (mean difference, 5.9 points; 95% CI, 0.36 to 11.43; p=.03), and placebo/sham tDCS (mean difference, 11.5 points; 95% CI, 6.03 to 17.10; $p\_$.001). Analysis of tDCS + placebo medication vs. sertraline + sham tDCS demonstrated comparable efficacies (mean difference, 2.6 points; 95% CI, _2.90 to 8.13; p=.35). Use of tDCS +placebo medication (but not sertraline + sham tDCS) was superior to placebo + sham tDCS. Common adverse effects did not differ between interventions, except for skin redness on the scalp in active tDCS (p=.03).

Active vs. sham tDCS was significantly superior for all outcomes (Odds Ratios for response and remission were, respectively, 1.63; 95% CI = 1.26–2.12 and 2.50; 95% CI = 1.26–2.49). There were 7 episodes of treatment-emergent mania or hypomania, five of which occurred in the combined treatment group.

Comment.

This is largest trial to date of tDCS in depression; the factorial (2X2) design enables the authors to address issues of monotherapy vs. co-initiation of tDCS and antidepressant medication. The combination of sertraline and tDCS (initiated simultaneously) was associated with better outcomes than in other arms of the trial, this may be a pointer to particular efficacy of co-initiated combination therapy. The choice of primary outcome endpoint at 6 weeks differs from all previous tDCS RCTs in depression, as it encompasses both the 2-week intensive treatment phase, and 2 fortnightly add-on tDCS sessions.

2.2.3.1.8.   Bennabi (2014)[285]

This study examined the utility of tDCS in treatment resistant depression. A total of 24 patients meeting diagnostic criteria for DSM-IV criteria for Major Depressive Disorder, with a baseline MADRS score greater than 24, and meeting stage II criteria (or above) for treatment resistance. All patients received a constant dose of escitalopram (10-20mg/d) over 4 weeks prior to tDCS treatment initiation. Concomitant treatment with benzodiazepine and/or second-generation antipsychotics was allowed. Exclusion criteria included bipolar depression, psychotic features, neurological/ severe organic disease or treatment with First Generation Antipsychotics. Subjects were randomised to receive active (*N*=12) or sham (*N*=12) tDCS. Both subjects and clinicians were blinded to allocation. Ten treatment sessions were delivered over five consecutive days (two treatments per day). The anode was placed over the Left DLPFC, and the cathode over the right supraorbital area. Active tDCS was delivered using 2mA intensity for 30 minutes per session. Sham tDCS was delivered using identical settings, but the current

was gradually ramped down to zero mA. Depression severity was assessed using the HDRS21 (primary outcome measure), MADRS, and BDI. Response was defined as a decrease of at least 50% from baseline HDRS score, remission was defined as a HDRS score of 8 or less. There was no significant difference between active and sham tDCS in the change in HDRS score (p=0.69): immediately after the course ended, in the active tDCS group there were 3 subjects who responded and 2 who met criteria for remission. In the sham tDCS group there was 1 responder and no remitters. The authors comment that one subject developed mania and withdrew from the study, but they do not mention the group allocation for this subject. There were no other serious adverse events. The authors do not supply information about the degree of blinding integrity.

Comment. This study is limited by the small number of subjects. There is considerable level of treatment resistance, and similarly to the Blumberger study[284], subjects started a new antidepressant 4 weeks prior to the trial, and may have experienced medication-related effects.

2.2.3.1.9.   Segrave (2014)[276]

This was the first study to examine the use of tDCS in conjunction with Cognitive Control Training (CCT), a type of neurocognitive training , which like tDCS is aimed at activating the DLPFC. The rationale for combining both modalities is that there is evidence to suggest that there are more pronounced functional outcomes when tDCS is applied to active brain regions, rather than to areas at rest.[286-288]. CCT is a novel therapeutic modality for depression, aimed at activation of the DLPFC through two targeted cognitive activities designed in reference to functional imaging data, to

activate the DLPFC[276]: the first, a modified Wells Attentional Training (WAT) paradigm is a guided auditory process, directing attention through phases of focused attention, switching attention and divided attention; the second, is a modified Paced Serial Addition Task (PASAT), a mental arithmetic task. 27 subjects with DSM-IV Major Depressive Episode, whose baseline MADRS score was greater than 18 took part in this study. Exclusion criteria included: Lifetime history of neurological illness, mania, hypomania PTSD or psychosis, and substance use disorders in the year prior to study initiation. Subjects were either not prescribed antidepressant medication, or were stable on the same dose for at least 4 weeks prior to study initiation. Subjects were randomised to receive five sessions of either: 1.tDCS + CCT (N=9), or 2.sham tDCS + CCT (N=9), or 3. tDCS + sham CCT (N=9). Participants and raters were blinded to allocation, but the operator was aware of allocation. Sessions were delivered on 5 consecutive working days. Active tDCS was delivered for 24 minutes per session using current setting of 2.0 mA. Sham tDCS was delivered using a 2minute fade out period. CCT started 2 minutes after initiation of tDCS session. The anode was placed over Left DLPFC (F3 according to the 10-20 EEG system), the cathode over the lateral aspect of the Right orbit (F8 according to the 10-20 EEG system). Results: there were no dropouts during the course of treatment, tDCS was well tolerated and no serious adverse events were reported. There was a significant difference between the three arms in respect of the change in MADRS scores over time $F_{(4,48)}$=4.63 (p=0.03). Post-hoc analysis demonstrates significant reduction in MADRS scores for subjects in the sham-tDCS+CCT group (p=0.02), and in the tDCS+sham-CCT group (p=0.04); there was a trend towards significance in the tDCS+CCT group (p=0.06). At 3-week follow-up, only the tDCS+CCT group showed significant difference from baseline MADRS scores (p<0.001). There

were no significant differences in response rates immediately following the 5-treatment course (*p*=0.08). At 3 week follow up, there was a significant difference in response rates (*p*=0.04): tDCS+CCT 44%, sham tDCS+CCT 11%, tDCS+sham CCT 0%. For the purpose of this meta-analysis, we included only 2 of the 3 study arms – comparing tDCS+CCT vs. sham tDCS+CCT.

Comment. An interesting finding was the delayed onset of maximal therapeutic efficacy of the tDCS+CCT combination. This is not consistent with a previous study of the tDCS+CCT combination in healthy individuals[289]. This finding supports the inclusion of follow-up phases in future tDCS studies.

2.2.3.1.10. Brunoni (2014)[290]

This is the second study examining the combination of tDCS and CCT for the treatment of depression. Subjects fulfilled criteria for DSM-IV major depressive episode, with a baseline HDRS score > 21; the age range was 18-65. Exclusion criteria included: 1. Bipolar disorder, substance use disorders, schizophrenia, personality disorders, and other co-morbid psychiatric disorders apart from anxiety disorders. 2. Neurological conditions. 3. Patient prescribed antipsychotics or tricyclic antidepressants. All subjects were taking antidepressant medication (SSRI or SNRI) with no dose changes for at least six weeks prior to Study initiation. Subjects were randomised to 10 treatments on consecutive working days, consisting of either: 1. CCT+sham tDCS (n=17) or 2. CCT+tDCS (*N*=20). The primary endpoint was 4 weeks post initiation (2 weeks of active treatment and 2 weeks of follow-up). tDCS parameters used were: 30 minutes per session at 2mA current setting, Anode over Left DLPFC (F3 according to the 10-20 EEG

system), Cathode over Right DLPFC (F4 according to the 10-20 EEG system). Sham tDCS used 30 sec ramp-in, 30 sec active stimulation and 15 sec ramp-out. The CCT intervention included only the modified PASAT, and was delivered during the final 15 minutes of each tDCS session. Results; tDCS was well-tolerated and no adverse effects were reported. There was 1 drop out from the active tDCS+CCT and 3 drop outs from the sham tDCS+CCT groups during the active treatment phase. There were no statistically significant differences between the groups in respect of the primary outcome measure. Both groups demonstrated similar reductions of HDRS scores at week 2 (p=0.91) and at week 4 (p=0.71). There were no statistically significant differences in categorical response and remission rates at week 2 or at week 4. Older subjects demonstrated a stronger additional effect of tDCS when combined with CCT. There were no additional improvement in depressive symptoms during the 2-week follow-up period (in contrast to the findings in the previous CCT+tDCS study[276].

Comment. This study demonstrated no statistically significant differences between active vs. sham tDCS, when added to a course of CCT in subjects with unipolar depression. The lack of significant difference at the end of the active treatment phase is consistent with the previous CCT+tDCS study[276]; however, in this study there was also no difference after a 2 week follow-up period.

2.2.3.2. Published meta-analyses

Our search of the literature identified 3 published meta-analyses of tDCS in depression. Their main design features and those of our own meta-analysis are summarised in Table 2.2.

Meta analysis	Date range	Trials	N subjects	Power to detect "small" effect (g=0.30)	Precision (pooled confidence interval width)	Outcome measures	Other analyses
Kalu et al., 2012	01/01/1998-05/2011	6	Active tDCS: 96 Sham tDCS: 80	0.50	1.06	Continuous- mean change in depression rating scale scores	Publication bias
Berlim et al., 2013	01/07/ 1998-20/08/2012	6	Active tDCS: 103 Sham tDCS: 97	0.55		Categorical- response and remission rates	Publication bias
Shiozawa et al., 2014	2006-31/01/2014	7	Active tDCS: 167 Sham tDCS: 152	0.76	0.66	Both categorical and continuous outcome measures	Publication bias
This meta-analysis	01/01/1995-30/04/2015	10 **(43% increase)**	Active tDCS: 206 Sham tDCS: 187 **(23% increase)**	0.84 **(11% increase)**	0.52 **(21% increase)**	Both categorical and continuous outcome measures	Publication bias Power analyses Precision analyses

Table 2.2: Comparison of previously published and current meta-analyses of tDCS in depression

2.2.3.2.1.   Kalu et al (2012) [259]

The first systematic review and meta-analysis of tDCS in the treatment of depression included randomized parallel or cross-over studies of active tDCS vs. sham tDCS controls, double blind allocation to treatment, and outcome measures including a clinician-rated depressive symptom severity scale (HDRS or MADRS), and an account of the change (%) in symptom severity. Six randomized controlled trials including a total of 96 patients in active tDCS arms and 80 patients in sham tDCS arms were included [274,277-279,281,282]. Depressive symptom severity was reduced by a weighted mean of 28.9% (14.6-60%). A weighted mean of 21.8% (range: 0-80%) of RCT participants receiving active tDCS experienced categorical response (50% symptom severity reduction), and 6.1% (range 0-23%) experienced symptomatic remission (HDRS score < 8 or MADRS score <11). The wide variability between studies may have reflected differing levels of treatment resistance, concomitant medication use, and variability in delivery of tDCS between trials. The pooled estimate of effect size (Hedges' g) for depressive severity reduction between active and sham tDCS was 0.74 (Z=2.76, p=0.006, 95% confidence interval 0.21–1.27). All four studies, which included 1-month follow-up data, reported that the reduction in symptom severity with treatment was maintained (and in 2 studies increased in magnitude) at follow-up. No significant correlations were found with baseline symptom severity, concomitant antidepressant use, stimulation current strength or total number of sessions. The most common adverse effects in both active and sham tDCS study-arms were headaches, and

local itchiness and/or redness at electrode sites. Skin-lesions were associated with the use of tap-water instead of saline solution for electrode preparation. Four cases of tDCS-associated hypomania were reported - 3 in published trials [278,282,291] and one in an on-going trial [292]. The authors conclude that tDCS is a potentially effective treatment for depression, but acknowledge limitations such as the small number of available studies, small number of participants, and heterogeneity in participant populations and treatment parameters. The authors called for large-scale studies with longer follow-up periods in more representative participant groups.

2.2.3.2.2. Berlim et al (2013) meta analysis [260]

The second meta-analysis of tDCS in the treatment of MDD investigated the utility of tDCS using response and remission rates as outcome measures. Systematic review searched for publications which fulfilled the following inclusion criteria: randomised, sham-controlled, double-blind, parallel or cross-over design with at least 5 subjects randomised to each study arm; participants aged 18-75, with a primary diagnosis of DSM-IV or ICD-10 Major Depressive Episode (MDE) of a unipolar or bipolar nature; treated with at least 5 sessions of tDCS, at an intensity of at least 1mA, with the anode over the left DLPFC, tDCS being administered as either monotherapy or augmentation strategy; publications in the English language. Exclusion criteria included enrolment of subjects with subsets of depression (e.g. post-partum or vascular depression); contemporaneous initiation of tDCS and another treatment for depression (e.g. antidepressant medication); and studies, which did not report response and/or remission rates. The literature search yielded 6 RCTs, including 5 of

the 6 trials incorporated by Kalu et al. [259] in their meta-analysis [274,278,279,281,282]: together with a more recent RCT [284]. Overall, the dataset included 200 subjects with MDE, 103 of whom were randomised to active tDCS. Subjects received a 10.8 +/- 3.76 sessions of tDCS. The cohort had failed to respond to a mean of 2.36 +/- 1.19 trials of antidepressants. There was no significant difference in baseline depression severity between the active and sham tDCS groups (p=0.66). Using drop-out rates to assess overall treatment acceptability, there were no significant differences in drop-out rates between active (4.8%) and sham (5.1%) tDCS (p=0.86). There was no significant difference in blinding integrity between active and sham tDCS (p=0.41); 44.1% of active tDCS participants correctly guessed treatment allocation at study end, vs. 53.7% of sham tDCS participants. The overall rates of response and remission were not significantly different between the active and sham tDCS groups: response rates were 23.2% for active tDCS vs. 12.4% for sham tDCS, pooled OR = 1.97 (95% CI =0.85-4.56; p=0.11); and remission rates were 12.2% for active tDCS vs. 5.4% for sham tDCS, pooled OR = 2.13 (95% CI = 0.64-7.06; p=0.22). Active tDCS significantly out-performed sham tDCS when used as a monotherapy for MDE: OR =7.54 (95% CI = 1.630-34.8; p=0.01). There was no association between number of treatment sessions (5 vs. 10 treatments) or electrical current used (1 vs. 2 mA) and treatment efficacy. The authors highlighted limitations, including small sample sizes, little or no follow-up after treatment protocol is ended, and no differentiation between effects in unipolar and bipolar depression.

2.2.3.2.3. Shiozawa et al (2014) [261]

The most recent meta-analysis of tDCS for depression was conducted by the same group that published the second meta-analysis [260]. This meta-analysis aimed to improve on previous meta-analyses by addressing two main areas: First, previous meta-analyses had utilised different methodologies for calculating effect size: the Kalu meta-analysis[259] used continuous depression severity scores, whereas the Berlim meta-analysis[260] used categorical response/remission rates. Second, previous meta-analyses had not includes data from the biggest tDCS for depression trial to date[275]. The inclusion criteria were: randomised, sham-controlled trials, providing data including continuous depression scores and categorical response + remission rates. The meta-analysis includes 7 RCTs [274,275,277,278,281,282,284], (N=259 participants). Active tDCS significantly outperformed sham-tDCS on continuous depression scores (g=0.37; CI 0.04-0.7). Odds Ratios for response and remission were respectively 1.63; 95% CI=1.26-2.12 and 2.50; 95% CI=1.26-2.49.

2.2.3.3. Discussion of previous meta-analyses

The three published meta-analyses of tDCS for depression present inconsistent conclusions; this may be due to a number of factors: First, choice of outcome measures for calculating effect sizes - the 2012 meta-analysis [259] used percentage change in depressive rating scale scores from baseline to endpoint, whereas the 2013 meta-analysis [260] used categorical response and/or remission rates, dichotomous outcomes which effectively raise the threshold for demonstrating differential effects between active and sham interventions. The 2014 meta-analysis [261] utilises both approaches: its conclusions are

aligned with previous findings in respect of continuous depression rating scale data [259], but are at odds with previous conclusions regarding response/remission rates [260] - this may be due to the different studies included in this meta-analysis (in particular, the inclusion of the large 2013 trial [275]). Second, the 2013 meta-analysis includes a study, which recruited participants with highly treatment-resistant depression (including those whose depression failed to respond to ECT, who were excluded from earlier trials). Third, there were an increased proportion of studies involving tDCS augmentation vs. Monotherapy in the 2013 meta-analysis: tDCS trials in which participants are allowed to continue antidepressant medication. This may limit the apparent effectiveness available to be demonstrated by tDCS, as the scope for active tDCS to increase the response and/or remission rates may be reduced when subjects are concurrently treated with antidepressant medication; some medications (for instance anticonvulsants) may actually reduce the efficacy of tDCS; and there may be an increased degree of treatment refractoriness in patients recruited to these trials. The 2014 meta-analysis [261] included large RCT [275] in which ADM and tDCS were co-initiated – this group outperformed other arms of the trial (as well as giving rise to most manic switches), which may indicate a particular increase in antidepressant efficacy when tDCS & Sertraline are co-initiated.

2.2.3.4. Narrative analysis of recent randomized controlled trials and meta-analyses of tDCS in depression

The body of evidence examining the use of tDCS in depression has grown significantly in recent years, to include 10 RCTs and 3 meta-analyses.

As regards efficacy, RCTs have yielded mixed results: 4 RCTs[277] show a statistically significant advantage for active tDCS over sham tDCS ('positive RCTs'), Whereas 6 RCTs[276,281,284,285,290] failed to demonstrate a significant separation between active and sham treatments ('failed RCTs') (table 2.3).

Factors associated with these differential outcomes can be divided into participant-related factors and tDCS-related factors (table 2.3)

Participant-related factors include treatment-resistance level, and concurrent treatment with medication or with CCT. Using the mean number of antidepressant medication trials before starting tDCS as a measure of the mean level of treatment resistance in each cohort, it seems that 4 RCTs[276,281,284,285] recruited patients with higher levels of treatment resistance. The mean number of failed ADM trials in these studies was greater than 2 (compared to 1.0-1.7 in all other RCTs for which data was available). All these studies found no advantage of active over sham tDCS, whereas 3: out of 4 RCTs in which tDCS was used for less treatment-resistant depression found evidence for superiority of active over sham tDCS[274,275,282]. The available data suggest that active tDCS is more likely to outperform sham tDCS in patients who have failed less than two trials of ADM, than in those who have failed more than two trials of ADM. It is notable that a similar pattern is seen in antidepressant drug RCTs: compared to patients who have not been treated or who have failed one ADM trial, patients who have not responded to more than 2 trials of ADM demonstrate a significantly lower response rate to subsequent ADM trials [293]. As

regards concurrent medication usage, 2 out of 10 RCTs did not permit concurrent ADM use during the trial, both found significant superiority of active over sham tDCS[274,277,290]. The potential for additional improvement with tDCS in patients who are already prescribed an antidepressant or undergoing CCT may be limited, in comparison with patients who are receiving tDCS monotherapy. Antidepressants may also directly interfere with tDCS efficacy.

TDCS-related factors include current settings, number of tDCS sessions and session duration, as well as electrode placement. Of the 4 positive RCTs, three used the higher (2mA) rather than the lower (1mA) current setting. By contrast, of the six failed RCTs, two used the lower current setting (1mA) in all participants [278], or in some of them [281]. Palm et al.[281] compared the outcomes for participants who received tDCS at 1mA vs. 2mA and found there was no significant difference (p=0.38) between groups: however, this trial recruited participants with treatment resistant depression, in whom the difference in efficacy between current levels may not have been sufficiently great to be associated with significantly different treatment outcomes. As regards the number of sessions, the number of sessions used in RCTs ranges from 5 to 15. Of the four positive RCTs, one involved 5 treatment sessions, two involved 10 treatment sessions, and a single trial involved 15 sessions. The negative RCTs include 2 trials involving 5 sessions, three involving10 sessions, and one involving 15 sessions.

Of course, it may be that these factors influence the efficacy of tDCS in an additive fashion: among the 6 failed RCTs, 4 trials recruited participants with higher levels of treatment-resistance. The only failed RCT which reported recruiting participants with lower levels of treatment-resistance mean number of failed ADM courses <2) [278], combined a short course of tDCS (5 treatment sessions), with a low current setting (1mA).

Table 2.3: Participant-related factors, tDCS-related factors and tDCS trial depression outcomes:

Name	Diagnosis	N active, sham	Age (S.D.)	Gender % Female	Rating Scale	tDCS Current (mA)	tDCS Session Duration (min)	tDCS Montage: anode, cathode	Number of tDCS Sessions	Treatment resistance level	Con-current ADM	Con-current CCT	Depression outcome
Fregni 2006[277]	Unipolar	9,9	48.2 (10)	No data	MADRS	1.0	20	F3 , FP2	5	no data	No	No	Active>Sham
Boggio 2008[274]	Unipolar	21,10	49 (7.4)	67.5%	HDRS	2.0	20	F3 , FP2	10	1.7	No	No	Active>Sham
Loo 2010[278]	Unipolar	20,20	47.3 (11.3)	55%	MADRS	1.0	20	F3 , FP2	5	Active 1.0 Sham 1.7	Yes	No	Active=Sham
Palm 2011[281]	unipolar & bipolar	11,11	57 (12)	50%	HDRS	1.0 or 2.0	20	F3 , FP2	10	Active 2.9 Sham 2.91	Yes	No	Active=Sham
Loo 2012[282]	unipolar & bipolar	33,31	48.2 (12.5)	46.6%	MADRS	2.0	20	F3, F8	15	Active 1.71 Sham 1.79	Yes	No	Active>Sham
Blumberger 2012[284]	Unipolar	13,11	42.7 (11.6)	45.6%	HDRS	2.0	20	F3, F4	15	Active 4.3 Sham 4.1	Yes	No	Active=Sham
Brunoni 2013[275]	Unipolar	30,30	42 (12)	68%	MADRS	2.0	30	F3, F4	10+2	1.7	Yes	No	Active>Sham
Bennabi2014[37]	Unipolar	12,12	61.8 (16.3)	75%	HDRS	2.0	30	F3 , FP2	10 (5 days twice daily)	100% > 2	Yes	No	Active=Sham
Segrave 2014[276]	Unipolar	9,9	40.4 (14.5)	37%	MADRS	2.0	24	F3, F8	5	3.0	Yes	Yes	Active=Sham
Brunoni 2014[290]	Unipolar	20,17	18-65	70.2%	HDRS	2.0	30	F3, F4	10	35-41% > 2	Yes	Yes	Active=Sham

Depression outcome is a categorical statement as to whether active tDCS out-preformed sham tDCS in terms of depression outcome measures (p≤0.05), at the end of active treatment course. ADM=Antidepressant Medication. CCT=Cognitive Control Training. Treatment resistance indicated by number of ADM trials failed prior to tDCS

2.2.3.5.   Analysis of published meta-analysis efficacy factors

Consideration of the 3 published meta-analyses, provides the following efficacy-related insights:

2.2.3.5.1.   Kalu et al. 2012 [259].

This meta analysis utilised continuous outcome measures and showed that active tDCS was associated with significant reduction in symptom severity compared with sham tDCS. It found that meta-regression with participant related factors (baseline severity, concurrent treatment with antidepressant medication), and with tDCS-related factors (number of sessions, current settings) did not yield any significant correlations.

2.2.3.5.2.   Berlim et al. 2013 [260].

This meta-analysis utilised categorical response and remission outcome measures, and concluded that there was insufficient evidence to support tDCS as superior to placebo in achieving response or remission from depression. Neither participant-related, nor treatment-related factors were significantly correlated with differential treatment outcomes.

2.2.3.5.3.   Shiozawa et al. 2014[261].

This meta-analysis utilised both continuous and categorical outcome measures, and found that tDCS with statistically superior to sham tDCS in the treatment of depression In both outcome domains. Meta-regression of both categorical and continuous outcome measures did not yield any statistically significant correlation between participant-related or treatment-related factors and treatment outcomes.

2.2.3.5.4. Published meta-analysis safety and tolerability findings:

The three published meta-analyses contribute the following insights into safety and tolerability:

2.2.3.5.5. Kalu et al. 2012 [259].

The most common adverse effects reported by studies included in the systematic review were of a minor nature including headaches, itchiness and redness of skin underlying the electrodes. Skin lesions reported by Palm et al. 2011[294] were not observed once the electrodes were soaked in saline solution rather than tap water. The authors note that although adverse effects were more commonly reported in the active tDCS than in sham tDCS groups, the differences were not statistically significant. There were several reports of 'treatment emergent' hypomanic episodes.

2.2.3.5.6. Berlim et al. 2013 [260].

Dropout rates at study end did not demonstrate a statistically significant difference between active and sham tDCS groups. The authors did not comment on safety aspects.

2.2.3.5.7. Shiozawa et al. 2014[261].

No statistically significant differences were found between acceptability (as measured by dropout rates) of active versus sham tDCS.

2.2.4. Current Meta-Analysis results

2.2.4.1. Current Meta-Analysis: Continuous Treatment effects

Across all studies the combined treatment effect was significant and consistent with a medium effect size (_k_=11, _g_=0.30, 95% CI=[0.04, 0.57], _p_= .027)(see Figure 2.2a). The 'probability of superiority' metric [278] indicated a 62% chance that a randomly sampled individual receiving active tDCS would have a greater reduction in depressive symptoms than a randomly sampled individual receiving sham tDCS 95% CI [52% - 72%]. A 'leave one out' analysis revealed that removing Boggio et al (2008) [274], Loo et al (2012)[34], or the concurrent Sertraline group of Brunoni et al (2013)[27] would reduce the effect to non-significance (largest _p_ =.078, smallest _g_ = 0.23). The meta-analytic combination of effects yielded a valuable increase in precision. From the earliest study to the most recent, the margin of error (width of one arm of the confidence interval: MOE) decreased from 1.18 to 0.26. The test for heterogeneity was significant (Q(10) =19.27, _p_= .037) and the I^2 statistic indicated that 40% of the heterogeneity between studies could not be accounted for by sampling variability, justifying the use of a random effects model.

2.2.4.1.1. Fail-safe N analyses

The "fail safe N" calculation using the Rosenthal approach [270], revealed that 27 unpublished studies averaging null results would be required in order for the treatment effect to dip below significance (_a_=.05). Additionally, we used the Orwin fail-safe _N_ calculations [269] to estimate the number of unpublished studies averaging null results that would be required to reduce the effect size to a range of target levels, this data is plotted in Figure 2.2b.

2.2.4.1.2. Publication bias

A funnel plot of the outcomes is shown in Figure 2.2c. To examine the sensitivity of the data to publication bias we employed the nonparametric "trim and fill" method [295]. The procedure estimated that one study (on the left of the summary effect) could have been suppressed due to publication bias. Imputing this missing study and repeating the analyses marginally reduced the effect size, which has become statistically non-significant (k=12, d=0.22, 95% CI=[-0.11, 0.56], p = .195).

2.2.4.1.3. Precision and power analyses

By using our interval of effect size estimates (g=0.30, 95% CI [0.04 0.57]) as a plausible population estimate of the treatment effect, we estimated the sample size required for future trials to yield target levels of precision, expressed in terms of the maximum confidence interval width for g. All analyses were performed to provide a level of 99% assurance that the confidence interval would be sufficiently narrow. This data is plotted in Figure 2.2d.

For sample size planning, we estimated that for an individual study to detect the summary effect estimated by our meta analysis at the p = 0.05 level with 80% power, an N of at least 173 would be required in both the treatment and control group (with the N required to detect the upper and lower bound being 49 and 12693 respectively). These estimates by far exceed the mean sample size of the studies included in the meta analysis (N=18). These analyses suggest that the studies included in the analysis do not meet the criterion for adequate statistical power.

2.2.4.1.4.   Moderator analysis for continuous outcomes

A summary of the one-moderator models is shown in table 2.4. Treatment resistance level was removed due to the inconsistent reporting (for separate groups, or for all participants combined, or no exact values) which prevented a useful and informative component of this analysis. Meta regression did not detect any significant moderators of the treatment effect – no one-moderator model provided a better fit to the data than an empty (no moderator model). In a multiple regression model, we calculated the proportional contribution of each moderator to the overall R^2, collapsed across orderings of regressors. This revealed that concurrent ADMs and concurrent CCT were the most important predictors, accounting for 47% and 36% of the total variation explained respectively (Figure 2.2e). Closer examination of these factors revealed that samples who were not taking concurrent ADMs (g=0.71,95% CI [0.12 1.29], p= 0.019) had a larger treatment effect than those who were (g=0.18,95% CI [-0.16 0.51], p= 0.302). Similarly, samples that did not receive concurrent CCT (g=0.39,95% CI [0.13 0.65], p= 0.004) had a larger treatment effect than those who did (g=-0.20,95% CI [-0.82 0.41], p= 0.517). Treatment parameters (including number of sessions and current settings) were not found to be significant moderators. No interactions between moderators could be tested, due to empty cells in the model matrices.

Table 2.4: A summary of the calculated one-moderator models and associated significance levels.

Q_0 is the Wald-type chi squared value for the omnibus test of model coefficients. R^2 is the pseudo R^2. b_0 and b_1 are the slope and intercept respectively. (ISI=inter-session interval in days, ADM = Antidepressant Medication

Moderator	k	df	Qo	p	R^2	b_0	b_1
Current	11	1	0.50	.481	0.0	0.57	-0.30
Number of sessions	11	1	0.00	.964	0.0	0.28	0.00
Session length	11	1	014	.705	0.0	0.35	-0.11
Total tDCS time	11	1	0.07	.791	0.0	0.43	-0.00
Total current	11	1	0.41	.783	0.0	0.41	-0.00
ISI	11	1	0.50-	.481	0.0	0.26	0.30
Diagnosis	11	1	0.00	.995	0.0	0.31	-0.00
Concurrent ADMs	11	1	2.35	.125	0.0	0.70	-0.53
Concurrent CCT	11	1	3.03	.082	41.2	0.39	-0.59

)

2.2.4.2. Current Meta-Analysis: Categorical treatment effects (Response and Remission rates)

Data for response rates were available from 9 of the 11 effects (Figure 2.3a). The pooled _LOR_ for response was positive, but did not reach significance (_k_= 9, _LOR_ = 0.36, 95% CI [-0.16, 0.88], _p_ = 0.176), Heterogeneity between studies did not exceed that expected by chance (_Q (8)_ = 6.18, _p_= 0 .627) and the I^2 statistic indicated that only 0.86% of the heterogeneity could not be explained by sampling error. Cumulative meta-analysis revealed that the meta-analytic combination of effects yielded an increase of precision from an MOE of 1.71 to 0.52. Data for remission rates were available from 9 of the 11 effects (Figure 2.3b). Consistent with response rates, the pooled LOR for remission was positive, but did not reach significance (_k_= 9, _LOR_ = 0.25, 95% CI [-0.42, 0.91], _p_ = 0.468), Heterogeneity between studies did not exceed that expected by chance (_Q_(8)=4.68, _p_ = 0.791) and the I^2 statistic indicated that all heterogeneity could be attributed to sampling error. Cumulative meta-analysis revealed that the meta-analytic combination of effects yielded an increase of precision from an MOE of 2.97 to 0.66.

2.2.4.2.1. Fail safe N analyses

Owing to the non-significant summary effects for response and remission, we did not compute fail-safe N analyses based on a nominal significance level. Instead, we used the Orwin method to assess the impact of publication bias on effect size. These data are plotted in Figure 2.3c.

2.2.4.2.2. Publication bias

Analyses revealed that no studies were trimmed and filled on the opposite side of zero in either the response or remission meta-analyses. Furthermore, both funnel plots were broadly symmetrical, suggesting a low risk of publication bias (Figure 2.3d & e).

2.2.4.2.3. Moderator analysis for categorical outcomes

Meta regression revealed no statistically significant moderators of either response or remission rates.

2.2.4.3. Current meta-analysis: safety and tolerability:

Dropout rates were available from 9 studies (table 2.5) and were analysed in a random effects model using the log odds ratio as an effect size measure (effect sizes greater than 0 indicate a greater likelihood of dropout in the active relative to the sham tDCS group). The analysis revealed no significant differences in drop out rates (*k*= 9 *LOR* = 0.05, 95% CI= [-1.0, 1.10], *p* = 0.928).

Study	n-active	drop out rate due to adverse effects - active tDCS	n-control	drop out rate due to adverse effects - sham tDCS	p
Fregni 2006[29]	9	0	9	0	1
Boggio 2008[26]	21	0	19	0	1
Loo 2010[30]	20	0	20	0	1
Palm 2011[33]	11	0	11	0	1
Loo 2012[34]	33	1	31	1	.964
Blumberger 2012[36]	13	1	11	0	.558
Brunoni 2013[27]	60	3	60	1	.347
Brunoni 2014[42]	20	1	17	3	.245
Segrave 2014[28]	9	0	9	0	1

Table 2.5 : Drop out rates due to adverse events in blind phase of tDCS depression RCTs

2.2.5. Discussion

We carried out a meta analysis of 10 RCTs comparing active tDCS to sham tDCS, including 393 participants with major depressive episodes in the context of unipolar or bipolar disorders. tDCS was used as mono therapy or as adjunctive treatment for depression in conjunction with medication and/or Cognitive Control Training (CCT). Analysis of continuous outcomes - depression rating scale scores, demonstrates clear superiority of active tDCS over sham tDCS in the treatment of MDE. The combined treatment effect was significant and consistent with a medium effect size (k=11, g=0.30, 95% CI=[0.04, 0.57], p= .027) (Figure 2.2).

It is important to highlight the findings from our precision and power analyses: These indicate that for an individual study to detect the summary effect at the p =.05 level at 80% power, an N of at least 173 would be required in both the treatment and control group. The mean sample size of the studies included in the meta analysis is 18 - it is therefore likely that the RCTs included lacked sufficient statistical power. Given that an N of 173 is of considerable size, future studies may wish to focus on enhancing the precision of their interval based estimates within practical and financial constraints, rather than placing too much stock in conclusions based on null hypothesis significance testing. In this context, *a priori* power and precision analyses (and explicit reporting of these values) are recommended to enhance interpretation of data relating to tDCS efficacy in future RCTs. The issues surrounding power, in turn, also go some way towards providing a possible explanation for the lack of statistically significant differences between active and sham tDCS in the categorical Response and Remission analyses that we carried out. Categorical outcome measures are more robust and clinically meaningful, but require a larger sample size to demonstrate an effect. We

found that both response and remission rates showed a positive pooled LOR but failed to reach statistical significance. These findings are consistent with the findings from earlier meta-analyses: Kalu et al. (2012)[259] used continuous outcome measures and found a significant superiority of active over sham tDCS; whereas Berlim et al. (2013) [260], used categorical outcome measures and failed to demonstrate significant differences. The more recent meta analysis Shiozawa et al (2014)[261] used both types of outcome measures and demonstrated significant superiority of active over sham tDCS in both continuous and categorical outcome measures. This difference in findings is likely to be mainly due to the way we analysed data from the largest tDCS trial to date [275] – we decided to analyse the outcomes at week 2, immediately following the daily tDCS treatment phase – in contrast to analysing the outcomes at 6 weeks (the primary outcome measure for the trial), following 2 fortnightly "top-up" sessions. The rationale for our choice being that most other RCTs we included in the meta-analysis use an endpoint at the termination of the intensive tDCS treatment phase. The outcomes at week 6 demonstrate an added degree of separation between active and sham tDCS – this may have been sufficient to provide overall statistical significance to the categorical analysis. This accords with the findings from other tDCS trials[276,282,296], indicating that at follow up, participants' depression rating scales score continued to improve. We also decided to separately analyse the data from participants who received sertraline and those who received placebo medication in the Brunoni et al. 2013 trial[275] – this created two separate effects (hence although we included 10 RCTs, we analysed k=11 effects). Moderator analysis demonstrated that although no moderators reached statistical significance at the level of p=.05, concurrent use of antidepressant medication, and concurrent use of Cognitive Control Training were the

most important predictors, accounting for 47% and 36% of the total variation explained respectively (Figure 2.2e).

Samples who were not taking concurrent ADMs (g=0.71,95% CI[0.12 1.29], p=.019) had a larger treatment effect than those who were (g=0.18,95% CI[-0.16 0.51], p=.302). Similarly, samples that did not receive concurrent CCT (g=0.39,95% CI[0.13 0.65], p=.004) had a larger treatment effect than those who did (g=-0.20,95% CI[-0.82 0.41], p=.517). It is of note, that the largest trial to date [275] included a group of participants, in whom tDCS and pharmacological antidepressant therapy were *concurrently initiated* – the authors report that this group did particularly well in terms of depression outcomes, and go on to postulate that simultaneous initiation of tDCS and ADM may confer added benefits in patients with MDE. One should note that these participants were treated with a low dose of antidepressant medication (sertraline 50mg daily). Insufficient data prevented us from conclusively analysing the effect of treatment-resistance level as a moderator of tDCS outcomes. More trials are needed in order to improve the precision of moderator analyses, this is currently limited due to small k. The evidence suggests tDCS has a good safety and acceptability profile, with only mild adverse effects reported in most trials. There were no statistically significant differences in the dropout rates between active and sham tDCS groups in any of the RCTs. The only serious adverse event recorded in published tDCS RCTs was a case of suicide, which was considered by the authors as unlikely to be directly related to tDCS [278]. Early reports of tDCS trials included descriptions of burns to the skin underlying scalp electrodes, but this has not been reported since researchers started using physiological saline rather than water to soak electrodes prior to use [297]. Several cases of tDCS-associated hypomanic episodes have been reported in the literature [259,261,275].

This meta-analysis offers several improvements compared to previous publications (Table 2.2): The literature search extends up to April 2015, and therefore captures 3 new RCTs (43% increase), which were not included in previous meta-analyses. This enabled us to increase the number of subjects by 23%. We were able to assess the effect of adding tDCS to CCT, this was not previously meta-analytically addressed. The power to detect "small" effects is increased, as is the precision (21% improvement in pooled confidence interval width). We also performed power and precision analyses to directly inform future research in terms of sample size planning.

Limitations: The main limitation of this meta analysis is the low number of participants in most trials included. As demonstrated by our precision and power calculations, all but one of these trials [275] are probably underpowered. This is likely to explain the lack of separation between active and sham tDCS in terms of categorical response and remission outcomes; as well as limiting the number of moderators reaching statistical significance. There is a lack of evidence regarding longer-term outcomes of tDCS in the acute and maintenance treatment of depression.
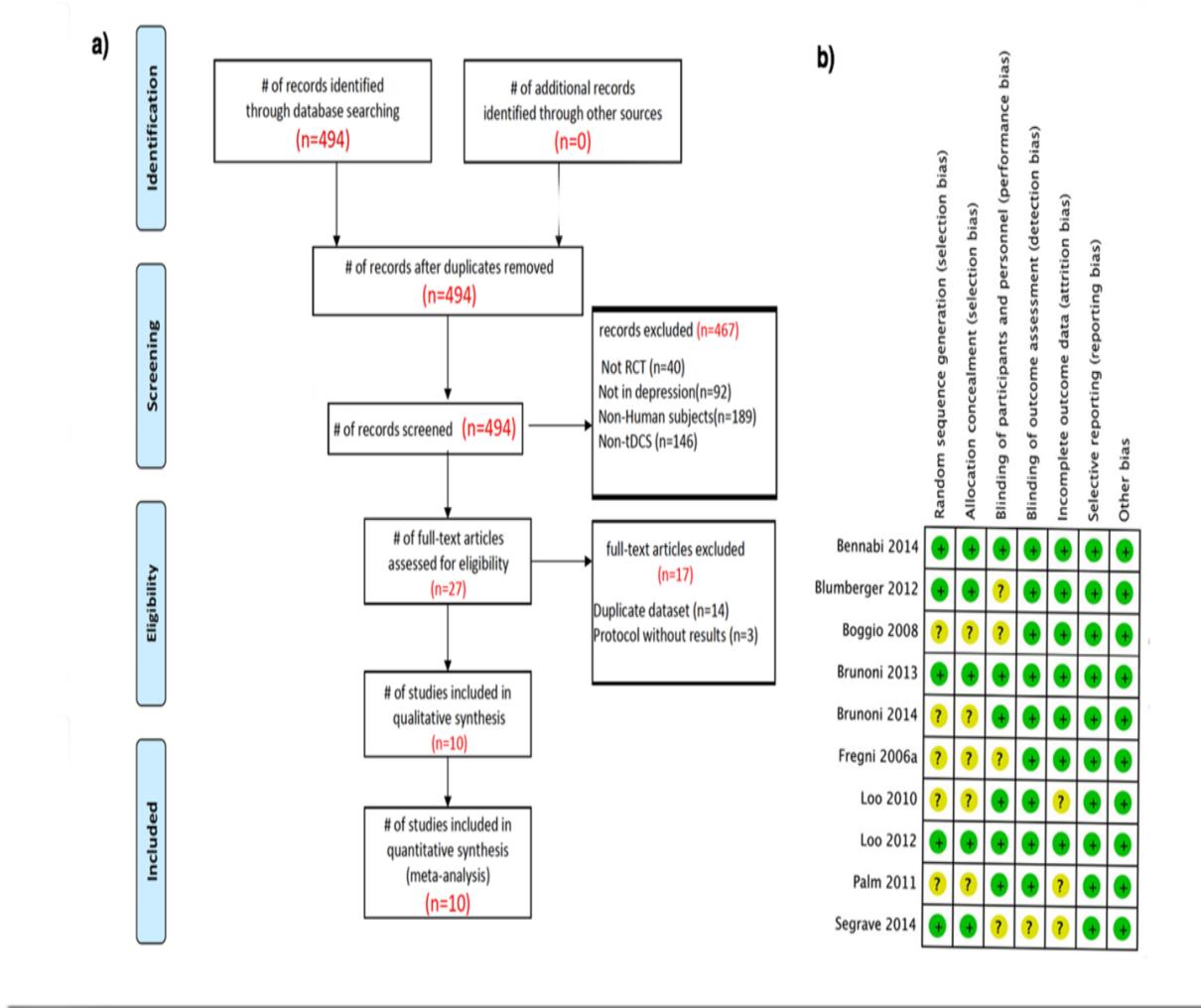
Figure 2.1 Study selection and quality assurance: (a) PRISMA[263] study selection flowchart for our systematic review and meta-analysis. (b) Summary of risk of bias in line with the Cochrane Collaboration's tool for assessing risk of bias in randomised trials[273].

RCT = Randomised Controlled Trial. Green circles represent low risk, yellow circles represent unclear risk of bias. The summary was generated using Cochrane's Review Manager software (RevMan5.3) - http://tech.cochrane.org/revm
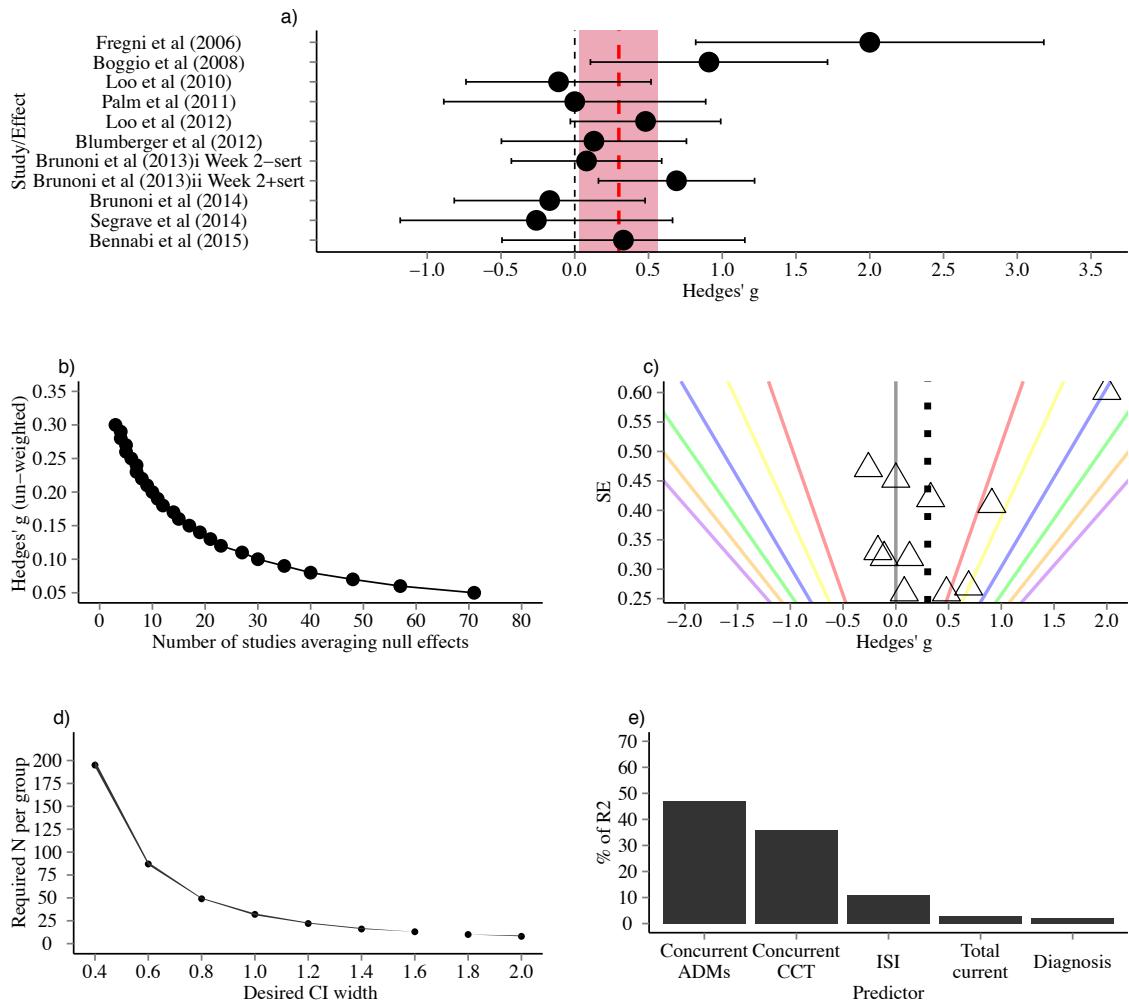
Figure 2.2 (a) Forest plot of effect sizes for active versus sham treatment. (b) Orwin fail-safe N analyses (c) Funnel plot. (d) Outcome of precision analyses (e) Relative importance of each moderator.

In (a) Error bars are 95% confidence intervals. Dotted red line depicts pooled effect. Shaded red region is 95% confidence interval. In (c) Dotted line is the pooled effect size, coloured lines are p values (e.g. red=.05, yellow = .01, blue= .001). In (d) for instance, to have 99% assurance that a 95% CI will be less than 0.8, approximately 50 participants per group would be required. Note that total tDCS time, amplitude, session length and number of sessions were removed due to multi-colinearity. Total current is instead included to summarize these variables.
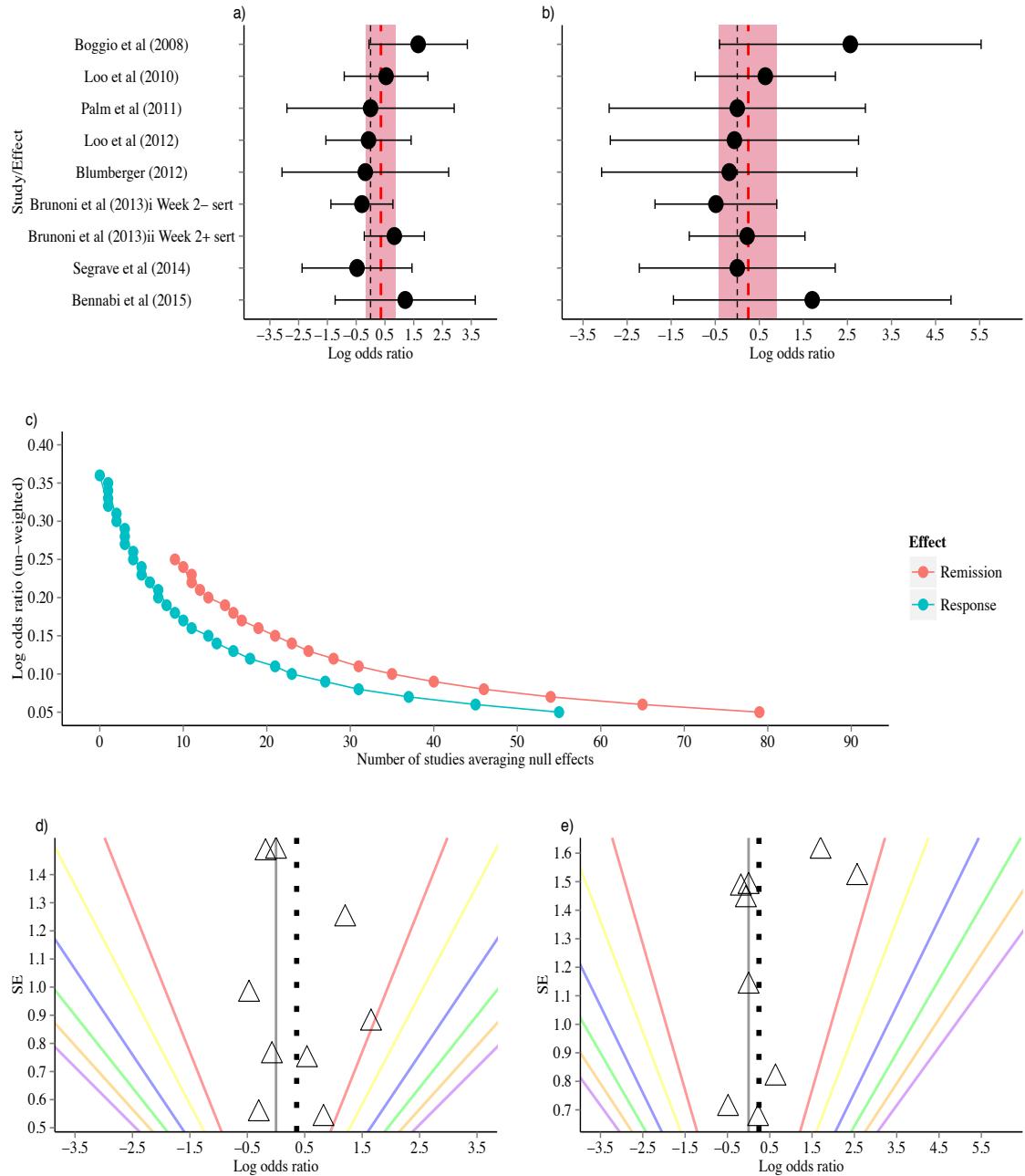
Figure 2.3. *(a) Forest plot of effect sizes for active versus sham treatment: response rates. (b) Forest plot of effect sizes for active versus sham treatment: remission rates. (c) Orwin fail-safe N analyses (d) Funnel plot: response rates. (e) Funnel plot: remission rates.*

In (a) & (b): Error bars are 95% confidence intervals. Dotted red line depicts pooled effect. Shaded red region is 95% confidence interval. In (d) & (e): Dotted line is the pooled effect size, coloured lines are p values (e.g. red=.05, yellow = .01, blue= .001).

2.3. Transcranial direct current stimulation (tDCS) for anxiety disorders:

2.3.1. tDCS in anxiety disorders - Literature search

In contrast to the growing evidence base for the use of transcranial direct current stimulation in depression, there is relatively little published research about its use in anxiety disorders[298].

I performed a search of the PubMed database using the following search strategy:

(((((("direct"[Title/Abstract]) AND "stimulation"[Title/Abstract])) OR "tdcs"[Title/Abstract])) AND ("anx*"[Title/Abstract] OR "panic*"[Title/Abstract] OR "OCD" [Title/Abstract] OR "obsess*"[Title/Abstract] OR "compuls*"[Title/Abstract] OR "PTSD" [Title/Abstract] OR "traum*"[Title/Abstract] OR "Phob*"[Title/Abstract]) . The date range extended up to April 30th, 2015. I also scrutinized the reference lists in published reviews of tDCS in anxiety disorders and articles listed as citing these reviews. Inclusion criteria used were: English language publications; Patient population with anxiety disorders; tDCS as monotherapy or augmentation therapy for treatment of anxiety. Exclusion criteria used were: Studies in animals; studies in non-clinical populations; trials of treatments for disorders other than anxiety disorders; trials of interventions other than tDCS; duplicated data-sets.

2.3.1.1. tDCS in anxiety disorders – results of literature search

This literature search yielded no randomised sham-controlled trials of tDCS in anxiety disorders. I found reports describing the use of tDCS for treatment of Generalised

Anxiety Disorder (GAD), Panic Disorder (PD), Post Traumatic Stress Disorder (PTSD) and Obsessive-Compulsive Disorder (OCD).

2.3.1.1.1. tDCS for generalised anxiety disorder (GAD)[299]:

The search yielded one case report describing the treatment of a 58-year-old woman with a three-year history of generalised anxiety disorder, which failed to improve despite several courses of pharmacological therapy. The patient was not depressed - her $HDRS_{17}$ score was 7. She was treated with a course of 15 once-daily sessions of tDCS (five days per week). Each tDCS session lasted 30 minutes and the current setting was 2.0 mA. The anode was placed over the left deltoid (extracephalic position), and the cathode over the right dorsolateral prefrontal cortex (DLPFC). This montage was selected in order to target the Right DLPFC in isolation rather than to balance DLPFC activation between the two hemispheres, as per the standard depression montage. The evidence supporting the use of this montage in anxiety disorders derives from recent rTMS studies[300,301]: Low frequency rTMS stimulation (which, like cathodal tDCS causes diminished activation of underlying tissue), was associated with improved symptoms in patients with GAD.

The patient's anxiety symptoms, as rated on the generalised anxiety disorder 7-item scale (GAD_7) and the Becks Anxiety Inventory (BAI) resolved during the course of the treatment. She became asymptomatic, and remained so at 30 day follow-up.

2.3.1.1.2. tDCS for Panic disorder (PD):

The search yielded one case report[302] describing the treatment of a 44 year old woman with Panic Disorder (PD). Several courses of high-dose pharmacological monotherapy

treatments (venlafaxine, sertraline, amitriptyline, and quetiapine) failed to provide significant improvement of her symptoms, and caused significant adverse effect, which in turn caused her to discontinue medication. She did not present with comorbid depression. The patient was treated with tDCS using the following protocol: montage - cathode over the right dorsolateral prefrontal cortex, and anode over the left deltoid (Extracephalic). This montage was based on rTMS findings, indicating that slow stimulation over the right dorsolateral prefrontal cortex, which like cathodal tDCS, is aimed at reducing the excitability of the underlying cortex, was associated with improved anxiety symptoms[300]. Each session lasted 30 minutes; the current setting was 2.0 mA. 10 treatments were delivered on consecutive working days. Anxiety was rated using the Hamilton anxiety rating Scale (HARS). The patient experienced full remission by day 10, and remained in remission on day 30. No significant adverse effects were reported, apart from transient erythema at the stimulation sites.

2.3.1.1.3. tDCS for obsessive-compulsive disorder (OCD):

Narayanaswamy & Jose (2014)[303] described 2 cases of successful use of adjunct tDCS for treatment of SSRI-resistant OCD:

The first patient was a 39 year old woman with a history of five years of DSM-V OCD, resistant to treatment with high dose SSRIs, she was unable to engage with CBT due to intolerable anxiety. Baseline rating scale scores were: YBOCS=25; CGI-S=6; HDRS=12; HARS=23. The second patient was a 24-year-old man with a history of three years of DSM-V OCD with comorbidity social anxiety disorder and mild depression. OCD symptoms did not improve despite several courses of treatment with high-dose SSRIs and clomipramine. Rating Scale scores at baseline were: YBOCS=30; CGI-S=6; HDRS=11;

HARS=10. Both patients were treated with tDCS - the anode was placed over the left pre-supplementary and supplementary motor areas (pre-SMA/SMA), and the cathode over the right supra-orbital area. Twice daily, 20-minute sessions were administered for 10 days using current setting of 2.0 mA. Medications remained unchanged. Patient 1 was prescribed sertraline 300 mg/day; patient 2 was prescribed sertraline 250 mg/day. Both patients improved significantly during this course of tDCS. Post-tDCS rating Scale scores for patient one were: YBOCS=15 (40% reduction); HDRS=7; HARS = 15. Post-tDCS rating Scale scores for patient two were: YBOCS=16 (46.7% reduction); HDRS=3; HARS = 3.

Volpato et al. (2013)[304] describe a case of OCD sequentially treated with rTMS and tDCS (10 active and 10 sham sessions of each modality; 20 min sessions using 2.0 mA; the montage was: cathode over F3, anode on posterior neck). Neither modality was associated with significant improvement in obsessive-compulsive symptoms, but tDCS (and not rTMS) was associated with improvement in anxiety and depression. It is interesting to note that pre-treatment fMRI demonstrated hemispheric asymmetry with relative left sided hyper-activation in the right anterior neural circuits. tDCS was more effective than rTMS in correcting this imbalance.

D'Urso et al. (2014)[305], submitted a report of a 33 year old woman with treatment-resistant OCD who received 10 sessions of tDCS (2.0 mA for 20 minutes; Anode over pre supplementary motor area (pre-SMA) and cathode on the right deltoid, followed by 10 sessions of tDCS delivered with similar parameters but with reversed polarity. OCD symptoms were assessed using the Y-BOCS. At the end of the anodal treatment phase,

OCD symptoms were significantly worse. the patient improved when treatment polarity was reversed to cathodal stimulation over the pre-SMA region. This pattern suggests that pre-SMA hyper-function may be associated with OCD symptoms.

Mondino et al. (2015)[306] report the case of a 52 year old woman with OCD, who failed to respond to several pharmacological therapies (paroxetine, fluoxetine, venlafaxine, clomipramine, and aripiprazole) as well as to a course of Cognitive Behavioural Therapy (CBT). She was treated with 10 sessions of tDCS delivered twice daily for 5 days. The cathode was placed over the left Orbito-Frontal Cortex (OFC), above FP1 according to the 10-20 international EEG system. The anode was placed over the right occipital cortex, above O2 according to the 10-20 international EEG system. Stimulation was delivered for 20 minutes per session using a current setting of 2.0 mA. The patient continued taking her regular medication (duloxetine 60 mg/d lithium carbonate 800 mg/d and risperidone 4mg/d); the medication had not ben changed for at least 3 months prior to initiating tDCS. YBOCS score at baseline was 36.5. Immediately after completing the course of tDCS, the YBOCS score was 36, but 1 month later, the YBOCS score reduced by 26% to 27. This finding is in line with the findings of rTMS study using low-frequency rTMS[307] (which, like cathodal tDCS is associated with reduced cortical tissue excitability), this concluded that low-frequency rTMS of the left OFC produced significant but time-limited improvement in OCD patients compared to sham treatment[307].

2.3.1.1.4. tDCS for post-traumatic stress disorder (PTSD):

Impairment of working memory is an associated feature of Post Traumatic Stress Disorder (PTSD)[308], and is also a feature of mild cognitive impairment (MCI)[309,310]. A pilot, open-label, uncontrolled study[311] examined the utility of combining working memory training with tDCS, in four male participants (age range: 55-65) with a very longstanding (many years) diagnosis of PTSD, who also had deficits in working memory. The participants were offered 5 weekly sessions of tDCS, delivered for 10 min per session at a current setting of 1.0mA. The anode was placed over the Left DLPFC (F3 on 10:20 system) and the cathode over the Right Supraorbital area. The are similar parameters to those described by Fregni et al. in their working memory publication[244], in which they showed that tDCS was associated with enhanced working memory. Participants were subsequently offered five weekly sessions of computerised working memory training (each lasting 30-45 min). Results: Participants demonstrated significant improvement across a range of working memory cognitive and emotional measures. These changes were also associated with normalization of PTSD-associated EEG abnormalities in Event Related Potentials (ERPs) in relation to novelty response ($p<0.05$). The authors commented that this pilot study was promising and suggested that the use of tDCS and computer based working memory training for people with PTSD warranted further investigation.

These results are in line with the evidence for left DLPFC anodal tDCS in depression, and also with rTMS evidence that high frequency (excitatory) rTMS over the right DLPFC is associated with reduced anxiety[312].

2.4. Summary and conclusions

This chapter described the evidence base for tDCS as a treatment for depression and for anxiety disorders. The evidence base in depression is developed enough to allow a meta-analytic approach aimed at amalgamating the evidence from a range of RCTs, whose findings were sometimes contradictory. Based on the current evidence regarding tDCS for the treatment of depression, the following conclusions may be drawn: First, tDCS may represent an effective treatment option for patients presenting with major depressive episodes. Second, tDCS offers a generally acceptable tolerability profile, which may make it a useful alternative to antidepressant medication in patients who do not wish to take medication and for those who cannot tolerate antidepressant medication. Third, the current body of evidence does not support the use of tDCS in treatment resistant depression. Fourth, the current body of evidence does not support the use of tDCS as an add-on augmentation treatment for depressed patient who are already taking an antidepressant or undergoing Cognitive Control Training (CCT). However, there may be an advantage for concurrently initiating treatment with an antidepressant and tDCS. Further research is needed, in particular, involving larger sample sized over longer periods of treatment. The meta-analysis was published in a peer-reviewed journal, and served as a basis for the Royal College of Psychiatrists' position statement regarding the role of tDCS in the treatment of depression.

In contrast to depression, the evidence base for tDCS in anxiety disorders is currently under-developed. In view of the strong evidence for efficacy, acceptability, and safety of tDCS in depression; of the preliminary evidence of positive effects of tDCS in several anxiety disorders; and of the theoretical factors suggesting that tDCS may modulate

important anxiety-related cognitive and emotional factors, further research is necessary to clarify the potential role of tDCS in the treatment of anxiety disorders.

Chapters 6 and 7 of this thesis describe 2 studies of tDCS in healthy volunteers: the first study examines the effects of active vs. sham tDCS on attention network performance as measured using the ANT; the second study assesses the effects of active vs. sham tDCS on threat evaluation using the anti-saccade task in healthy volunteers inhaling 7.5% CO_2.

3. CHAPTER 3: FOCUSED ATTENTION AND OPEN MONITORING MEDITATION TRAINING AND ATTENTION NETWORKS FUNCTION

3.1. Introduction

3.1.1. Mindfulness

Mindfulness-based interventions are increasingly recognised as treatment options for a range of symptoms and disorders (see section 1.3.2 for a detailed discussion). Two main modalities of mindfulness-based interventions are described in the literature: Mindfulness Based Stress Reduction (MBSR), and Mindfulness Based Cognitive Therapy (MBCT). Other therapeutic approaches linked to mindfulness-based ideas, or which employ meditation as part of the intervention include Dialectical Behavior Therapy (DBT)[30], Acceptance and Commitment Therapy (ACT)[313], and Relapse Prevention (RP)[314]. The core feature of mindfulness as practiced in the context of therapeutic interventions is the development of a particular kind of attentional quality – practitioners are trained to pay attention '*on purpose, in the present moment and non-judgmentally*'[314] (see chapter 1 for a detailed discussion of mindfulness).

3.1.1.1. Mindfulness and anxiety

The evidence base for the efficacy of mindfulness-based interventions in anxiety is developing (see section 1.3.4 for a detailed discussion). Relatively little known about the mechanisms of action underlying these effects. There are several potential gains to be made by clarifying the mechanisms by which mindfulness-based interventions bring

about improvement in anxiety. First, understanding the mechanisms may facilitate the development of more streamlined approaches, doing away with 'non-essential' intervention components, thereby potentially shortening and simplifying the intervention. Second, clearly identifying the 'active ingredients' may allow the development of more potent mindfulness-based interventions. Third, understanding the mechanisms by which mindfulness-based interventions work in anxiety may enable the transfer of some of these mechanisms to strengthen existing non-mindfulness-based therapeutic approaches, and the development of novel treatments for anxiety.

3.1.1.2. Mindfulness – focused attention (FA) and open monitoring (OM)

Theoretical models of mindfulness (see chapter 1 for detailed discussion) highlight the role of attention training as a core component of mindfulness interventions: attention is one of Shapiro's three mindfulness axioms[200]; and Holzel describes "attention regulation" as a key mindfulness component[207]. Mindfulness-based interventions can be divided into two broad attentional training categories: focused attention (FA), and open monitoring (OM)[316-319].

3.1.1.2.1. Focused attention (FA) training

FA practices[316] emphasise sustaining selective attention on a selected object of meditation. The meditator is instructed to concentrate their attention on the chosen 'object', to monitor their awareness, and when their attention wanders away from the object, to return the focus of attention back to this object. FA training commonly utilises narrow focused objects such as the sensation of the breath in a particular part of the body. The skills/functions practiced in FA training are[317]: 1. maintaining

prolonged, stable attention on a chosen object; 2. monitoring for distraction without destabilising attentional focus on the primary object; 3. disengaging from distractions; 4. redirecting attention to the primary object. These functions have been linked to neural networks involved in conflict monitoring (including DLPFC and dorsal ACC)[320-322], selective attention (Including temporoparietal junction, ventrolateral PFC, and FEF)[69], and sustained attention (including thalamus and right frontal and parietal regions)[323,324]. As FA skills develop, the practice becomes increasingly effortless, and gradually induces attentional control trait changes, whereby attention focuses, and rests more readily on any selected object[316].

3.1.1.2.2.   Open monitoring (OM) training:

OM practices[316] commonly begin by stabilising attention on a narrow focus, and then expanding the focus to include whatever comes up in the field of awareness from moment to moment. OM training develops the following skills: 1. attention devoid of particular focus on a primary object; 2. non-reactive monitoring. 3. non-reactive awareness of automatic cognitive / emotional elaborations of sensory stimuli. OM practice is characterised by an accepting, inclusive openness towards any sensory, affective, or cognitive experience presenting within the field of awareness[317,318]. Buddhist psychology regards all of these experiences as sensory experiences, as the ability to sense emotions, thoughts and images is regarded as another sensory modality[325]. There is evidence that OM practice is associated with improved conflict monitoring[210], reduced attentional blink, improved efficiency of resource allocation to targets presented serially[326], and more distributed attentional focus[327]. As practice matures, it becomes increasingly effortless, and a 'non-grasping' trait develops

alongside a less reactive, more sensitive awareness of one's internal and external environment.

3.1.2. Attention – the three-network model

Attention is a complex phenomenon which includes several attentional components[90]. A number of sub-cortical and cortical networks interact and give rise to the group of processes underpinning attention[91]. In 1971 Posner and Boise put forward an early version of a three-network model[92]. This model maintains that there exist three demarcated attentional networks, each with its own structural and functional characteristics. These three networks can be viewed as aspects of an attentional organ system, incorporating histological, neuroanatomical, and functional components[97]. Different three-network models have been proposed over time[98,99], describing parallel, but not identical entities with different names[91]. The three attentional networks proposed by these models are currently termed: 'alerting', 'orienting', and 'executive'[100]. A detailed description of these networks is provided in section 1.1.3.7.

3.1.3. The Attention Network Test (ANT)

The Attention Network Test (ANT) is a computerised reaction time test first described by Fan and colleagues in 2002[100]. The ANT uses a combined cued reaction time[109] and flanker tasks[130] to independently measure the performance (Response Time and Error Rate) of the alerting, orienting and executive attention networks. The ANT version used in this study was modified to allow assessment of meditation effects on attention

network performance changes in response to neutral and negative word cues, in addition to non-word cues. This allows examination of mindfulness effects on processing of threat-related and negatively-valenced stimuli. This version is described in section 3.3.4.1.1.

3.1.4. Anxiety and attention

Anxiety is associated with distinct attentional patterns – these include reduced attentional control in the presence of negatively valenced and threatening stimuli[328], hypervigilance to threat and negatively valenced stimuli[329] and increased distractibility[328,330]. The attention control theory of anxiety describes a disruption of attentional resource-allocation balance, from 'top-down' (goal directed) towards 'bottom-up' (stimulus driven) attention[331]. These patterns may be associated with dysfunctional attention-control mechanisms underpinning pathological phenomena such as worry, anxiety and rumination. fMRI evidence lends support to the idea that anxiety is associated with reduced activity in frontal and/or prefrontal attention-regulating circuits[332].

The spectrum of anxiety is often subdivided into state and trait anxiety. The effects of these subtypes of anxiety on attention have been proposed to be different[136] – in that state anxiety enhances the threat valence of a stimulus, whereas trait anxiety causes attention to be directed consistently towards potential sources of threat. State anxiety is triggered by situational factors, so is likely to be associated with bottom-up processes, whereas trait anxiety is related to personality factors and therefore likely to

be more associated with top-down processes[73]. There is some evidence demonstrating that trait anxiety is associated with reduced executive control performance on the ANT: Pancheco-Unguetti and colleagues used a modified form of the ANT (ANT Interactions ANT-I) to test subjects with high vs. low trait anxiety scores[73]. High trait anxiety subjects demonstrated significant deficiencies in executive control network performance. State anxiety showed associations with orienting network and alerting network over-functioning. Pancheco-Unguetti and colleagues[74] compared performance on the ANT-I between patients with anxiety disorders and healthy controls. Anxiety disorders were associated with executive attentional network dysfunction and with reduced efficiency in attentional disengagement from invalid cues – including emotionally neutral cues. Han and colleagues showed that adolescents with comorbid depression and anxiety disorder demonstrated a faster orienting response on the ANT when compared to depressed adolescents without comorbid anxiety disorder[138].

3.1.5. Mindfulness and attention

Mindfulness training is directly concerned with developing Attentional skills, beginning with the development of concentration - the ability to maintain prolonged attentional focus on a particular object despite internal and external distractions, and continuing throughout the course of mindfulness training[207]. Section 1.3.6 provides a detailed overview of the effects of mindfulness on measures of attention.

3.2. Aims

This study compares the effects of two types of meditation training (FA and OM) vs. a relaxation control intervention, on attention network performance in meditation-naive

healthy volunteers, using the pre vs. post intervention ANT as the primary outcome measure. We used the modified (emotional) ANT in order to investigate the effects of mindfulness training on neutral/negative word cues.

We predicted that[333]:

- Both FA and OM training would be associated with post vs. pre-intervention improvement in executive control (but not in orienting or alerting) attention network performance.

- The relaxation control intervention would not be associated with significant changes in attention network performance.

- Due to its direct training approach to distractor non-engagement and to concentration on a primary attentional object, we predicted that FA training would be associated with a larger effect on ANT executive control performance, compared to OM training.

- Due to threat-related processing biases, the modified (emotional) ANT would demonstrate emotional-valence-related changes in orienting network performance due to spatial hypervigilance.

- AF and OM interventions would increase these spatial hypervigilance-related orienting network effects[208,209].

- OM would have a greater effect than AF on spatial hypervigilance-related orienting network effects due to its direct training approach to attentional-openness towards all stimuli.

3.3. Method

3.3.1. Participants

76 students from the University of Southampton (66 women and 10 men, all mindfulness novices) were recruited, having responded to advertised offers of course credit / money for taking part in the study. Participants were randomised to one of three experimental intervention groups: 1. Focused attention (FA) training (n=24); 2. Open monitoring (OM) training (n=25); 3. Relaxation control group (n=27). 3 of the participants randomised to the Relaxation control group were found to be high trait-anxiety outliers on the STAI (as determined using a box-plot in SPSS); they were excluded from the analysis (see figure 3.1) in order to prevent significant trait-anxiety baseline differences between the groups. Our sample size of 20+ participants per group provides 80% power at an alpha level of 5% to detect a medium-large effect size (Choen's d = 0.5 - 0.8), based on reviews of the effects of mindfulness on cognitive function (e.g. Chiesa et al. 2011[334]).
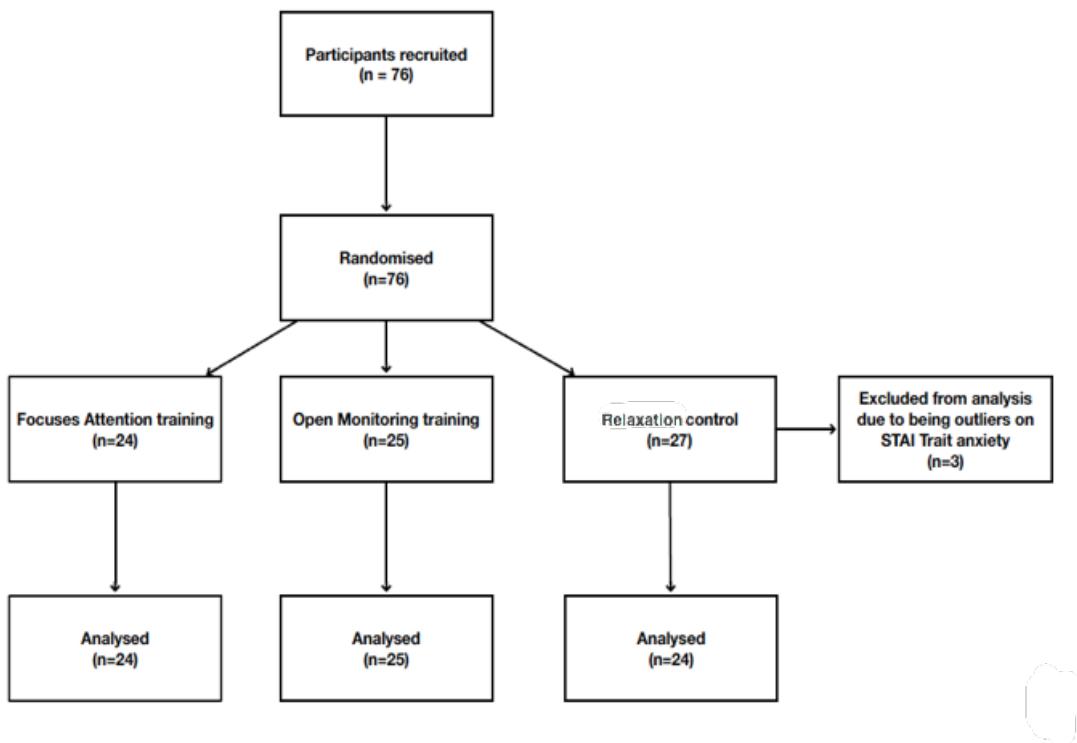
Figure 3.1: Consort diagram showing study design and progress of subjects through the trial.

3.3.2. Study design and workflow

The study was approved by the University of Southampton's research ethics and research governance committees (See figure 3.2 for study workflow). Participants were recruited using advertisements offering course credits / money in exchange for study participation. The only exclusion criterion was self-reported prior experience with mindfulness meditation. Participants underwent standard briefing and informed consent was obtained. Participants completed a set of baseline rating scales (see below for details) including the state-trait anxiety inventory (STAI)[335], attention control scale (ACS)[114], mindful attention awareness scale (MAAS)[336], Penn state worry questionnaire (PSWQ)[337], and Spielberger state anxiety inventory (SSAI)[335]. Participants went on to complete a baseline modified attention network test (ANT)[100]. (see below). Training

sessions were held on the Highfield campus (University of Southampton). The FA and OM intervention groups underwent three 60-minute training sessions over 10 days, and were also instructed to practice daily using an online audio mp3 containing a 10-minute guided meditation (FA or OM, depending on group allocation). The relaxation control group did not receive training sessions. Post-training assessment sessions consisted of repeated rating scales, and a modified attention network test – they occurred 3 weeks after the initial test-session (M = 21.5 days; SD = 4.3 Days). The interval between initial and final test sessions did not significantly differ between groups [$F_{(2, 70)} < .41$, $p = 0.66$]. Participants allocated to OM / FA groups undertook a 10-minute practice (guided by the same audio MP3 they used for homework) immediately before completing the final modified-ANT. Participants in the control group were instructed to sit quietly and relax for 10 minutes before completing the modified ANT.
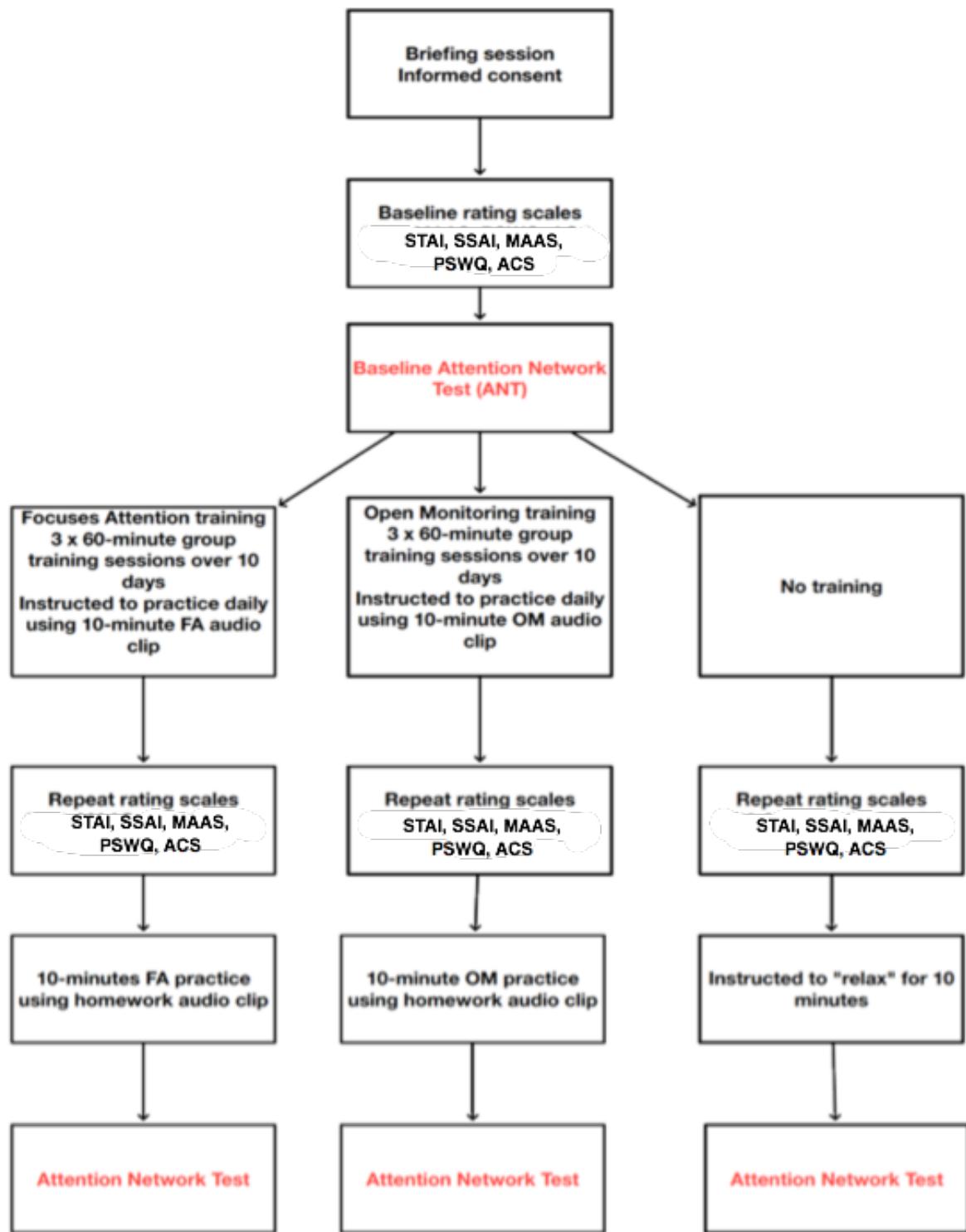
Figure 3.2: Procedural diagram describing Study design and workflow.

3.3.3. Experimental interventions

FA and OM training consisted of three hour-long group-training sessions held over 10 days, and daily guided meditation practice using an audio MP3. I developed the training package based on my experience of mindfulness practice and of delivering mindfulness-based clinical interventions. I have been practicing mindfulness meditation since 1990, have accrued several thousand hours of personal practice, have attended many residential mindfulness retreats (including several retreats led by the founders of MBSR and MBCT), and have more than 10 years experience in delivering mindfulness-based clinical interventions in a variety of individual and group settings. My competence level has been assessed as level 5-6 on the mindfulness-adapted stages of competence scale[333,338]. Training sessions included a brief theoretical introduction to practice, followed by a 20-30 minute period of guided group practice. Participants were encouraged to engage in a group reflective discussion. The expectation of adherence to daily homework was emphasised. Homework practice consisted of once daily individual 10-minute practice guided by an online audio MP3, recorded by me. Participants were asked to report on their degree of compliance with daily homework at debrief.

3.3.3.1. Focused attention (FA) training

Similar training instructions were given during the group sessions and the guided audio MP3 daily practice:

"Finding a place where the sensations of the breath are particularly clear right now…at the tip of the nose, the back of the throat, the chest or the abdomen…. making a decision

to stay with this place for the duration of this exercise rather than moving awareness

from one place to another…turning awareness towards this place…allowing awareness

to settle on this point…allowing the mind to become comfortable here…maintaining this

focus, and when the mind wanders, gently returning the mind to this place…..when the

mind has wandered, lightly and firmly returning the focus to this place….really

examining the sensation of the breath, and making the focus of attention as fine and as

exact as possible….really pinpoint this one point where the breath is observed."

3.3.3.2.   Open monitoring (OM) training

Similar training instructions were given during the group sessions and the guided audio

MP3 daily practice; participants were encouraged to briefly stabilise their awareness by

using a short focused awareness stage, but rather than staying with focus, they were

instructed to open the focus of awareness:

"Allowing a sense of awareness of the breath and physical sensations in the body

generally to gradually expand…. allowing awareness to include any sounds, whatever

the eyes see, and perhaps any smells and tastes that may be present…. allowing all of

this to come within the field of awareness…sitting here, with all of this, perhaps

allowing the emotional tone, how
Any feelings right now, to become part of this field of awareness – whatever sense of

comfort or discomfort, any emotions present, allowing that to become part of the field of

awareness right now, noticing any changes that may occur…"

3.3.4. Outcome measures

3.3.4.1. Primary outcome measure

3.3.4.1.1. Attention Network Test (ANT):

The Attention Network Test (ANT) is a computerised reaction time test first described by Fan and colleagues in 2002[100]. The ANT uses a combined cued reaction time[109] and flanker tasks[130] to independently measure the performance (Response Time and Error Rate) of the alerting, orienting and executive attention networks. In this study we used a modified (Emotional) ANT, each trial consisting of the following sequence (figure 3.3 a):

- Fixation: a fixation-cross presented for 400-1600 msec.

- Cue: presented for 100 msec. This could be either a neutral word cue (e.g. "WORLD"), a negative word cue (e.g. "NASTY"), a non-word cue (e.g. "XXXXX"), or no-cue (figure 3.3 c).

  - Spatial cues: displayed either 1° above or below fixation cross, alerting to onset and orienting to location of target - all spatial cues were spatially congruent.

  - Double cues: displayed both above and below fixation point – alerting to target-onset, but not orienting to target location.

  - Centre cues: displayed in location of fixation cross - alerting to target-onset, but not orienting to target location.

  - No-cue: lead-up to target onset was 100 msec longer without any preceding cue.

- Target & flankers: Central arrow and 4 flanking distractor arrows. All flanking

arrows pointing in the same direction, either congruent or incongruent with central target arrow (figure 3.3 b). The arrows appear 400 msec after cue offset, and remain displayed until participant responds by pressing a button to indicate whether the target arrow pointed left or right.

Each participant was asked to complete 24 randomised practice trials, followed by 480 randomized experimental trials: 72 trials per trial-type on the standard ANT, and 24 trials per emotion x trial-type condition on the Emotional ANT. Trials were randomised and counterbalanced for flanker congruence, target direction and location across cue-types.

Attention network performance calculations were performed by comparing mean response times (RTs) (figure 3.3 d):

- Alerting effect $= \text{Mean RT}_{\text{(No cue trials)}} - \text{Mean RT}_{\text{(Double cue trials)}}$

- Orienting effect $= \text{Mean RT}_{\text{(Centre cue trials)}} - \text{Mean RT}_{\text{(Spatial cue trials)}}$

- Executive Control effect $= \text{Mean RT}_{\text{(Incongruent trials)}} - \text{Mean RT}_{\text{(Congruent trials)}}$

Higher executive control scores suggest worse executive control performance, as the mean RT difference between the simpler congruous and more challenging incongruous trials increases.

Alerting, orienting, and executive attention network scores were each separately entered into a mixed-design ANOVA with group (FA, OM, Control) as a between-subjects factor, and cue-type (no-cue, double-cue, centre-cue, spatial-cue) and time (pre-intervention, post-intervention) as within-subjects factors.
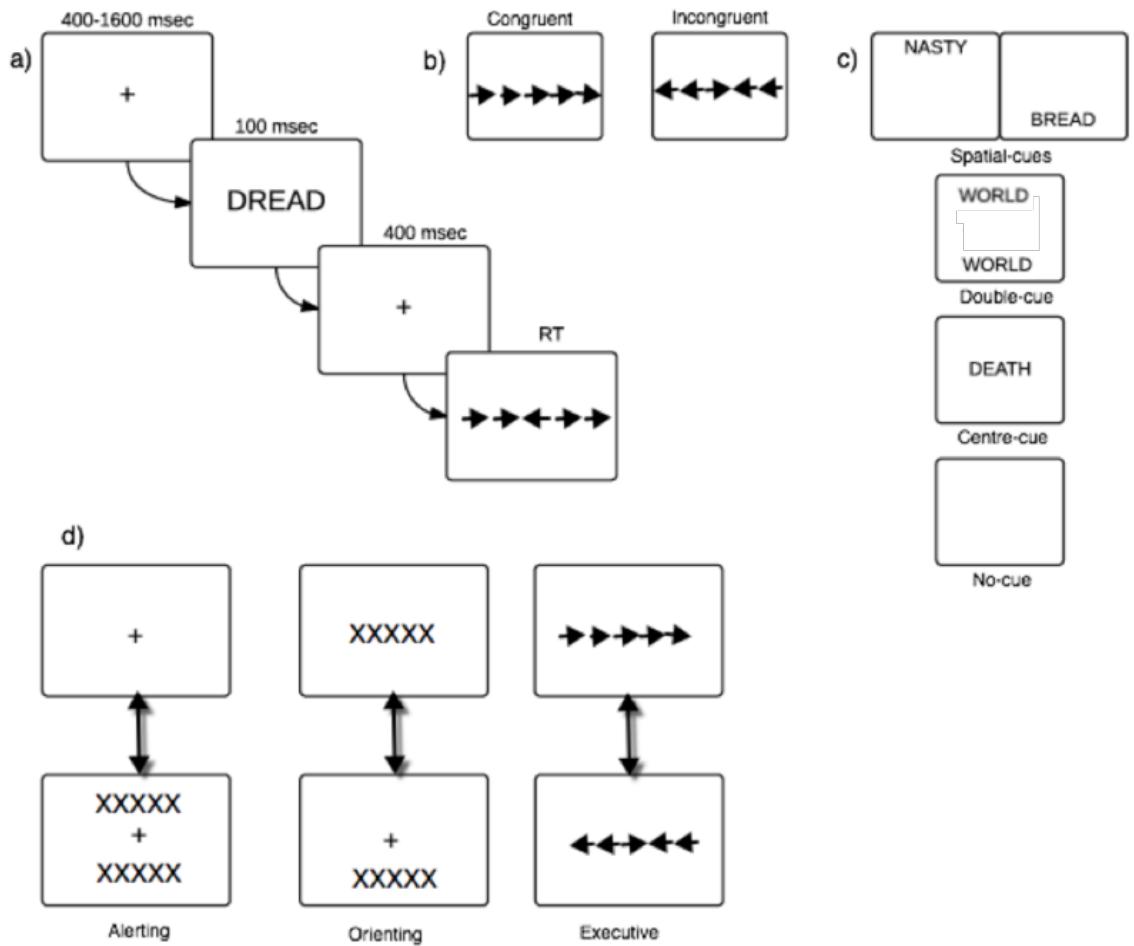
Figure 3.3 Modified (emotional) attention network test (ANT) a) ANT trial sequence; b) Target-flanker congruence; c) Cue types; D) Attention network performance calculations.
(Modified from Ainsworth, Eddershaw, Meron, Baldwin, & Garner 2013[333])

3.3.4.2. Secondary outcome measures:

3.3.4.2.1. Spielberger State-Trait Anxiety Inventory[335]

The Spielberger State-Trait Anxiety Inventory is a widely accepted instrument for measuring both state and trait anxiety. It has been translated into more than thirty languages, and cited in over 3000 studies[313]. The Spielberger State-Trait Anxiety Inventory consists of 40 items, 20 aimed at assessing trait anxiety (Spielberger Trait Anxiety Inventory - STAI), and 20 measuring state anxiety (Spielberger State Anxiety

Inventory - SSAI). Each item is a 4 point forced-choice Likert scale[314]. The state and trait scales each contain 2 factors: "anxiety absent" and "anxiety present". Trait anxiety is a tendency towards feeling anxiety, worry, discomfort, and stress; it describes a relatively stable characteristic or disposition, rather than a reaction to a particular set of conditions. The STAI addresses how subjects feel generally, commonly or usually. Items on the trait scale include statements such as: "I am content", "I have disturbing thoughts". Each item on the trait scale is scored: 1 almost never; 2 sometimes; 3 often; 4 almost always. State anxiety is a transient response to a set of conditions perceived as threatening or dangerous. It can include components such as autonomic arousal, and feelings of fear, discomfort, or nervousness. The SSAI measures how subjects feel "right now, at this moment"[314]. Items on the state scale include statements such as: "I am calm", "I am worried". Each item on the state scale is scored: 1 not at all; 2 somewhat; 3 moderately so; 4 very much so. Scores generated on each scale can range from 20 – 80, higher scores reflect more intense anxiety[314]. The Spielberger State-Trait Anxiety Inventory was found to have good internal consistency (average αs > 0.89)[314]. The test-retest reliability at different time points of the trait scale (STAI) is also good (average r = 0.88)[315]. The test-retest reliability of the state scale (SSAI) at different time-points is of course lower (r = 0.70).

3.3.4.2.2. Mindful attention awareness scale (MAAS)[336]

The MAAS is a 15-item questionnaire, inquiring about the presence or absence of attention in common everyday experiences (e.g. "I drive places on 'automatic pilot' and then wonder why I went there", and "I could be experiencing some emotion and not be conscious of it until some time later"). Each item on the MAAS invites response along a

6-point Likert scale ranging from 1: "Almost Always" to 6: "Almost Never". All items on the MAAS are constructed so that a higher score indicates a higher degree of dispositional mindfulness: in fact all items on the MAAS are formulated as mindlessness experiences – the reason being that these were thought to be more easily accessible to non-expert meditators[339]. The MAAS has been shown to have good internal consistency (Cronbach's α in range of 0.82 - 0.87)[336].

3.3.4.2.3. The Penn State Worry Questionnaire (PSWQ)[337]

The PSWQ is a well-established self-report instrument used to measure subjects' worry trait. The PSWQ contains 16 items relating to worry; each item is scored on a 5 point Likert scale ranging from 1 "Not at all typical of me" to 5 "Very typical of me". Eleven items are positively scored – for example: "I am always worrying about something", and 5 items are scored in reverse – for example: "I do not tend to worry about things". Higher scores are correlated with increased tendency to worry. PSWQ demonstrates good internal consistency (α = 0.90)[340], and test-retest reliability[341].

3.3.4.2.4. Attentional Control Scale (ACS)[114]

The ACS is a 20-item self-report measure assessing the ability to maintain attentional control in the presence of distractors such as environmental stimuli, concurrent tasks, and emotional states. The ACS presents items such as "When I am reading or studying, I am easily distracted if there are people talking in the same room", and "When trying to focus my attention on something, I have difficulty blocking out distracting thoughts"; it then asks subjects to rate themselves along a 4 point Likert frequency of experience scale ranging from 1 "Almost never" to 4 "Always". The ACS demonstrates good

internal consistency using a two factors Focusing (α = 0.82) and Shifting (α = 0.71) sub-scales[342].

3.4. Results

3.4.1. Participants

One-way ANOVA confirmed that the FA, OM, and control groups did not significantly differ in age [F (2, 73), = 0.47; p = 0.63] or gender ($\chi2$ = 1.61, p = 0.45). Comparing group distributions using box-plots across the entire sample, demonstrated that 3 participants were extreme outliers on STAI trait-anxiety, these participants had been randomised to the control group. We removed these participants from the analysis in order to avoid significant baseline differences in baseline trait anxiety levels between the groups (see consort diagram – *Figure 3.1)* Repeated one-way ANOVA following the removal of these 3 participants confirmed that the FA, OM, and control groups did not significantly differ in age [F(2, 70), = 0.40; p = 0.67] or gender ($\chi2$ = 1.45, p = 0.48). One way ANOVA confirmed that after removing the 3 outlier participants, there were no statistically significant differences between groups in their baseline self-reported measures of dispositional mindfulness as measured on the MAAS, trait-anxiety, or attention control (see table 2.1). Levels of anxiety in the cohort were in keeping with expected levels in healthy volunteers[343]; dispositional mindfulness levels on the MAAS were in keeping with normative values for young adults[344]

Table 3.1: Focused attention (FA), open monitoring (OM) and control group: baseline (pre-intervention) self-report measures.

	FA		OM		Control		F(2,70)	*p*
	n=24		n=25		n=24			
	M	SD	M	SD	M	SD		
STAI	40.1	8.5	42.2	10.7	44.7	*8.9*	*1.43*	*0.25*
SSAI	35.0	8.1	35.2	10.8	38.3	*9.7*	*0.89*	*0.41*
MAAS	54.1	10.4	59.0	10.1	55.2	*11.5*	*1.43*	*0.28*
ACS	46.0	5.3	46.2	8.0	45.0	*7.7*	*0.19*	*0.82*
PSWQ	46.6	11.5	48.8	13.0	43.6	*12.2*	*1.11*	*0.34*

STAI = Spielberger state-trait anxiety inventory; SSAI = Spielberger state anxiety inventory; MAAS = mindful attention awareness scale; ACS = attentional control scale; PSWQ = Penn state worry questionnaire

3.4.2. Effects of focused attention (FA) and open monitoring (OM) on attention network performance as measured on the ANT

Descriptive statistics for each condition within the ANT for each group are presented in table 3.2. Overall, reaction times (RTs) across all 3 groups were significantly shorter in the post-intervention vs. pre-intervention ANT [$F_{(1,70)} = 13.94$; $p < 0.001$]; however, the groups did not significantly differ in the extent of RT shortening (F<1). There were no significant effects of time, group, cue valence, nor their interaction on RTs, nor on error-rates.

Table 3.2: Mean ANT reaction time scores across groups for pre- and post-intervention test sessions.

(standard deviations in brackets).

			FA (n=24)		OM (n=25)		Control (n=24)	
			Pre	Post	Pre	Post	Pre	Post
Mean reaction times per cue-type x congruence x cue valence condition								
Central	Congruent	Negative	457.9 (48.4)	448.6 (60.1)	476.7 (54.7)	467.5 (73.6)	463.3 (75.3)	456.1 (76.6)
		Neutral	450.6 (38.8)	443.9 (43.7)	484.8 (66.7)	473.0 (74.0)	479.9 (99.6)	461.8 (76.2)
		XXXXX	456.0 (48.9)	444.1 (47.1)	484.7 (61.6)	469.0 (68.5)	471.5 (86.6)	459.8 (69.0)
	Incongruent	Negative	573.5 (73.9)	542.3 (76.3)	579.9 (65.6)	553.6 (88.0)	583.9 (94.6)	555.7 (85.8)
		Neutral	573.2 (64.9)	537.8 (67.2)	577.0 (69.5)	542.9 (68.3)	571.5 (96.5)	566.0 (106.2)
		XXXXX	571.6 (71.7)	540.5 (67.6)	587.7 (69.6)	544.1 (59.0)	588.6 (108.4)	563.8 (87.8)
Single	Congruent	Negative	448.3 (47.7)	427.1 (42.7)	473.0 (70.4)	448.8 (89.6)	457.0 (86.7)	444.8 (73.7)
		Neutral	440.4 (45.4)	431.7 (49.3)	464.0 (66.4)	441.5 (73.0)	458.9 (97.6)	439.2 (74.6)
		XXXXX	447.8 (48.6)	424.6 (38.7)	466.5 (78.8)	455.8 (89.2)	458.5 (86.6)	436.8 (74.2)
	Incongruent	Negative	540.2 (67.2)	515.2 (77.4)	546.3 (66.1)	512.2 (76.2)	551.8 (112.3)	526.8 (82.0)
		Neutral	534.7 (64.8)	496.6 (52.6)	555.4 (76.6)	510.7 (72.6)	550.4 (109.4)	525.1 (92.4)
		XXXXX	532.3 (65.2)	503.8 (53.3)	541.2 (72.7)	517.7 (65.8)	545.5 (107.0)	529.3 (92.4)
Double	Congruent	Negative	463.0 (48.0)	441.4 (45.6)	493.5 (66.1)	473.9 (82.7)	476.3 (80.2)	463.4 (80.2)
		Neutral	451.3 (41.6)	448.6 (49.6)	490.6 (62.5)	480.9 (95.1)	479.1 (86.6)	472.1 (74.1)
		XXXXX	462.4 (48.4)	443.4 (46.8)	496.4 (73.5)	473.7 (69.4)	490.6 (100.0)	46.1 (64.5)
	Incongruent	Negative	575.0 (73.0)	541.9 (76.9)	600.4 (81.6)	554.4 (64.7)	591.6 (118.3)	567.4 (92.0)
		Neutral	569.3 (66.1)	541.6 (70.5)	580.9 (63.3)	541.1 (57.5)	589.6 (97.0)	557.0 (102.7)
		XXXXX	580.5 (70.0)	541.7 (62.6)	583.8 (66.5)	549.5 (61.1)	594.3 (113.6)	573.9 (117.3)
No cue	Congruent		520.8 (46.6)	515.9 (61.1)	533.6 (70.9)	526.5 (71.4)	533.4 (103.0)	533.7 (71.5)
	Incongruent		610.6 (91.1)	575.6 (80.1)	605.7 (81.6)	595.5 (95.9)	617.0 (112.2)	601.2 (95.8)
Attention network function scores								
Executive Attention		Negative	106.5 (49.0)	94.1 (45.2)	94.5 (28.1)	76.6 (27.7)	103.5 (59.0)	95.8 (33.2)
		Neutral	111.6 (40.0)	83.9 (27.6)	94.6 (31.6)	66.4 (30.4)	93.2 (39.8)	92.0 (44.5)
		XXXXX	106.1 (38.2)	91.3 (28.9)	88.4 (29.1)	70.9 (24.8)	98.5 (41.1)	103.5 (39.4)
Orienting		Negative	21.5 (29.4)	24.3 (22.4)	18.6 (28.8)	30.1 (32.1)	20.3 (27.0)	21.1 (27.7)
		Neutral	24.3 (22.7)	26.7 (22.0)	21.7 (26.6)	31.9 (24.4)	19.7 (24.2)	32.3 (31.6)
		XXXXX	23.7 (25.9)	28.1 (20.6)	32.4 (30.9)	19.8 (26.2)	24.9 (28.9)	29.2 (32.7)
Alerting		Negative	46.7 (27.1)	54.1 (35.6)	22.7 (22.2)	46.8 (39.2)	46.1 (38.8)	52.1 (27.7)
		Neutral	55.4 (31.6)	50.7 (32.1)	29.4 (37.2)	50.0 (36.0)	46.5 (32.2)	54.7 (29.4)
		XXXXX	44.3 (30.5)	53.2 (33.1)	29.5 (32.6)	49.4 (40.5)	39.4 (35.8)	50.9 (32.8)

3.4.2.1. Alerting attention network performance

Alerting attention network performance was improved at post-intervention vs. pre-intervention as demonstrated by the significant main effect of time [$F(1,70) = 6.61$; $p = 0.01$; np2 = 0.03]. There was no significant effect of group [$F(2,70) = 1.46$; $p = 0.24$], cue-valence [$F(2,69) = 2.36$; $p = 0.10$], or interactions between group, time, and cue-valence [All F's < 1].

3.4.2.2. Orienting attention network performance

There were no significant effects of time [$F(1,70) = 2.35$; $p = 0.13$], or cue-valence $F(2,69) = 1.43$, $p = 0.25$] on orienting attention network performance. There were also no significant interaction effects between time and cue-valence [$F(2,69) = 1.60$, $p = 0.21$]; or interactions between time, cue-valence, and group [$F(4,140) = 1.67$; $p = 0.16$]. All other interactions were also non-significant [all F's <1].

3.4.2.3. Executive attention network performance

There was a significant main effect of time [$F(1,70) = 16.08$; $p < 0.001$; np^2 (partial eta squared) = 0.19]. This effect was subsumed under a significant time x group interaction [$F(2,70) = 3.32$; $p = 0.042$; $np^2 = 0.087$]. Although the groups did not significantly differ in baseline executive attention network performance [$F(2,70) = 1.097$; $p = 0.339$], the differences between the groups' executive attention network performance post-intervention were significant [$F(2,70) = 4.971$; $p = 0.01$]. Executive attention scores significantly improved from pre- to post-intervention in both the FA [$t(23) = 3.57$; $p = 0.002$] and OM [$t(24) = 3.83$; $p = 0.001$] groups, but there was no corresponding significant change in the control group [$t(23) = 0.19$; $p = 0.85$] (Figure 3.4). ANOVA did

not detect any other significant effects {cue-valence: [$F_{(1,70)}$ = 2.23, p = 0.12]; time x cue-valence [($F_{(2,69)}$ = 2.87; p = 0.06]; all other F's < 1}.
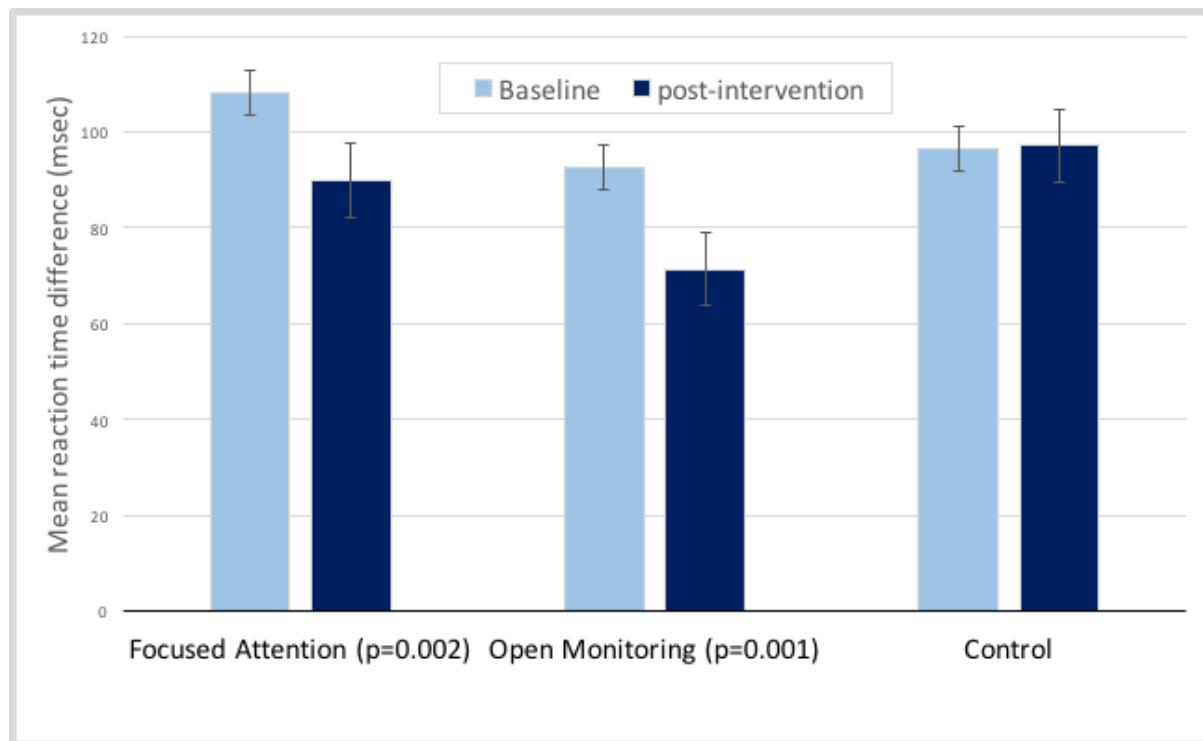
Figure 3.4 Executive attention network performance in the focussed attention (FA), open monitoring (OM) and Control groups at baseline and post-intervention.

Lower scores reflect improved performance.

3.4.3. Effects of focused attention (FA) and open monitoring (OM) on self-report measures of anxiety, mindfulness and attention-control

Mixed design ANOVA with group as between-subjects factor, and time as within-subjects factor detected no significant effects of time, group or time x group on the MAAS, STAI, or ACS (table 3.3)

Table 3.3: Focused attention (FA), open monitoring (OM) and control group: Pre- and post-intervention self-report measures.

		FA		OM		Control		F(2,70) Group x Time	p
		n=24		n=25		n=24			
		M	SD	M	SD	M	SD		
STAI	pre	40.1	8.5	42.2	10.7	44.7	*8.9*	*1.43*	*0.25*
	post	40.3	10.4	39.9	9.7	43.6	*10.1*	*0.99*	*0.38*
SSAI	pre	35.0	8.1	35.2	10.8	38.3	*9.7*	*0.89*	*0.41*
	post	33.9	9.5	33.2	9.7	37.2	*9.0*	*1.251*	*0.29*
MAAS	pre	54.1	10.4	59.0	10.1	55.2	11.5	1.43	0.28
	post	54.0	9.9	55.6	12.0	56.0	9.8	0.24	0.79
ACS	pre	46.0	5.3	46.2	8.0	45.0	7.7	0.19	0.82
	post	46.6	5.6	45.2	9.5	45.9	7.9	0.19	0.82
PSWQ	pre	46.6	11.5	48.8	13.0	43.6	12.2	1.11	0.34
	post	42.8	9.5	47.7	14.5	42.8	9.5	1.50	0.23

STAI = Spielberger state-trait anxiety inventory; SSAI = Spielberger state anxiety inventory; MAAS = mindful attention awareness scale; ACS = attentional control scale; PSWQ = Penn state worry questionnaire

3.4.4. Associations between attention network performance, attention control, mindfulness and anxiety

Baseline dispositional mindfulness (as reported on the MAAS) was positively correlated with baseline attention control (as reported on the ACS) (r= 0.43; p<0.001), and with baseline executive attention network performance (on the ANT) (r= - 0.25; p= 0.03). Baseline trait-anxiety was negatively correlated with dispositional mindfulness (r = -0.33; p= 0.005), and with attention control (r = -0.40, p= 0.001). We did not correct for multiple comparisons, however, a corrected significance level would be 0.01. There were no other significant correlations between attention network performance and self-report measures in this this study.

ANT score changes between baseline and post-intervention measurements were not significantly correlated with self report changes in dispositional mindfulness, anxiety or attention control (r's < 0.16) – therefore the change in ANT executive attention network scores observed in the FA and OM intervention groups was not related to changes in self-report anxiety, dispositional mindfulness or attention control. The duration of homework practice during the study period, as reported by participants, did not show significant correlation with the amount of change on self-report measure or change in ANT scores (r's<0.13, p's > 0.26). Baseline self report dispositional mindfulness, anxiety, and attention control did not predict ANT score changes (r's < 0.22). All self-report and ANT measures demonstrated high level of correlation between baseline and post-intervention scores, as would be predicted by their reportedly good test-retest reliability: MAAS r = 0.43, p < 0.001; ACS r = 0.40, p < 0.001; STAI-T r = 0.66, p <0.001; ANT executive network r = 0.63, p <0.001; ANT alerting network r = 0.40, p <0.005; ANT orienting network r = 0.25, p <0.05.

3.4.5. Adverse outcomes and side effects

No adverse outcomes or side effects were reported during this study.

3.5. Discussion

This is the first study to examine the effects of focused attention (FA) vs. open monitoring (OM) meditation training on attention network performance. Both FA and OM training were associated with a significant improvement in executive control attention network performance on the ANT. The ANT calculates the executive control effect by subtracting the mean RT in congruent trials from the mean RT in incongruent trials. This finding therefore reflects a significant reduction in distractor interference effect (difference between incongruent − congruent), consistent with improved attention control following FA and OM. This is likely to reflect an improvement in conflict resolution between task relevant and task irrelevant stimuli – a core executive control network function. This finding is in line with previously published evidence showing that mindfulness training is associated with improved executive control attention network performance on the ANT[208,210].

We predicted that FA training would have a greater effect on executive attention network performance, because FA training directly emphasises concentrated attention and distractor disengagement; however, there were no significant differences between FA and OM training effects on ANT executive control attention network scores. This may be due to OM group participants inadvertently practicing FA – Buddhist meditation traditions consider OM to be a more advanced meditation skill than FA, and

therefore do not usually teach it to meditation beginners[345]. The instructions we gave to OM group participants included an initial stage of FA practice at the beginning of each practice session – this is in line with traditional Buddhist meditation training, allowing attention to initially settle on a meditation object, before expanding the field of awareness to practice OM. Whenever participants found that they had become distracted, they temporarily returned to practice FA before widening their attentional focus to practice OM again. It may be that this method inadvertently contributed to improvement in FA skills among OM group participants, who may have found sustained OM too challenging to practice[316,317]. If this is the case, then it may be helpful to plan future studies utilising interventions that allow OM participants to develop more proficiency in OM, this may include more robust, longer instruction programmes, and longer practice sessions which may allow more scope for OM practice to stabilise and mature. Another potential explanation for the lack of separation between FA and OM effects on executive control attention network function is that OM training includes a core skill of disengagement from stimuli; in fact, in OM an individual is effectively training to disengage from all stimuli, allowing whatever arises in awareness to come and go. This skill can be conceptualised as overlapping with distractor disengagement as practiced in FA training. It is therefore possible that this is a mechanism mediating the effect of mindfulness training on improvement in executive functions.

The effects of FA and OM training on attention network performance in this study were specific to the executive network; there were no significant effects on alerting or orienting attention network performance. This selective effect is in line with neuroimaging evidence showing that mindfulness practice is associated with executive

control enhancements in response inhibition and that this is accompanied by increased activation of the prefrontal cortex[346], which is known to form part of the executive control network (see chapter 1 for a discussion of the anatomical distribution of attention networks). The correlation between baseline dispositional mindfulness (as measured using the MAAS) and pre-intervention executive control attention network performance, is in line with published evidence indicating that executive control performance correlates with meditation proficiency as demonstrated by the differences between experienced meditators and meditation-novices[208]. This study utilised a control group which did not receive any kind of alternative 'sham mindfulness' training. Finding an appropriate control condition for mindfulness studies is a challenging problem, and most studies to date utilised varieties of wait-list or no-practice control-groups, which make it difficult to differentiate between specific and non-specific effects of mindfulness-training[347]. In this study, it is unlikely that the observed effects were due to non-specific effects or to demand characteristics, as the effects were clearly focused on executive control network performance, did not generalise to other attention networks, and were not associated with self-report changes in dispositional mindfulness, anxiety or attention control. It is also important to note that motivation/engagement indicators such as global accuracy, RT-variability and overall response time did not significantly differ between groups.

We predicted that orienting network performance would improve in the FA and OM groups, with an effect of time x group x emotional valence of modified emotional ANT cues. There was no evidence of this effect. This would have been in line with evidence of threat-related attentional biases in anxiety, and of improvements in orienting network performance following a course of MBSR[208]. The study found no such effects.

A potential explanation is that our participants formed a low-anxiety cohort, in which the negatively-valenced word cues did not create sufficient levels of threat to trigger attentional biases[117]. This is also the likely explanation for the lack of hyper-vigilance to negative cues observed in this study. It is also possible that the mindfulness training we provided was not long enough – there is evidence that in contrast to an 8-week MBSR programme, shorter mindfulness-based interventions are not associated with orienting network changes[348]. Orienting ANT scores in this study also demonstrated lower test-retest reliability ($r = 0.25$), (c.f. $r = 0.63$ for executive attention performance) – this may indicate that the modified (Emotional) ANT is less sensitive to changes in orienting network performance.

This study found that whereas dispositional mindfulness (reflected in MAAS scores) and attention control (ACS scores) were positively correlated, they were both negatively correlated with trait-anxiety (STAI-T). This finding is in line with a correlational study[349], which found a similar pattern of correlation between trait-anxiety, attention control, and mindfulness. Core features of mindfulness, such as present-moment-focus, and non-judgemental acceptance, are at odds with anxiety characteristics like attentional biases and future-focused worry. Attention control has been found to mediate the relationship between mindfulness and anxiety[350], suggesting that the therapeutic effect of mindfulness-based interventions in anxiety may be at least partly due to enhanced attention control. This is further supported by a study in high-trait-anxiety healthy volunteers, which showed that while state-anxiety was associated with increased alerting and orienting network function, trait-anxiety is associated with specific deficiencies in the executive control network[73]. These anxiety-related executive control

attention network deficits are similar to the pattern described by the attentional control theory[24], and to those found in anti-saccade studies[72,122,143].

3.6. Limitations

There were several limitations to this study – (these are further discussed in section 8.5.2):

The lack of an active control group – the control group received no training, whereas the active intervention groups received 3 hours of face to face training, as well as daily homework practice.

The negative word cues used in the modified (Emotional) ANT task, were likely to be not distressing enough to elicit sufficient anxiety levels in this low-anxiety cohort.

There was no formal adherence measure in place, participants reported their level of adherence to homework at the end of the study, but there was no mechanism for tracking adherence contemporaneously.

All participants were meditation novices, and may have found the open monitoring (OM) method challenging, thereby repeatedly reverting to Focussed Attention (FA) practice

The mindfulness training intervention was not associated with significant effects on participants' levels of dispositional mindfulness as measured using the MAAS.

3.7. Implications and future prospects

This study demonstrated that a short intervention with either FA or OM meditation training is associated with significant improvements in ANT executive control attention network performance in healthy volunteers. Considering the important role of

attentional control deficits in anxiety-related cognitive processing difficulties, this finding lends further support to the therapeutic potential of brief mindfulness-based interventions in anxiety. This is further strengthened by the advantages of mindfulness-based interventions in terms of their acceptability, growing evidence base for efficacy and effectiveness, and the potential for delivering them in a variety of contexts including group-settings, and home-based training. Future research may consider whether there are particular advantages in targeting mindfulness interventions selectively at individuals who demonstrate particular anxiety-related deficits in executive control. For example, would a particular pattern of executive control dysfunction serve as a marker for mindfulness-related recovery? It might also be helpful to study whether early signs of improvement in executive control function might serve as predictors for subsequent symptomatic improvement in anxiety disorders. This study did not demonstrate the predicted effects of mindfulness training on orienting attention network performance in the presence of negatively-valenced cues on the modified emotional ANT. As discussed in the previous section, this may have been due to negative word cues in the modified ANT not being disturbing enough to generate sufficient threat perception in our low-trait-anxiety cohort. The next chapter will progress the investigation of mixed FA and OM mindfulness meditation training, by directly examining its effects on attention to threat using the anti-saccade task.

3.8. Funding

4. CHAPTER 4: EFFECTS OF MINDFULNESS MEDITATION TRAINING ON ATTENTION TO THREAT

4.1. Introduction

The previous chapter described a study, which demonstrated that a brief mindfulness meditation training intervention was associated with significant improvement in executive control attention network task performance in healthy volunteers. The previous study did not find significant differences between FA and OM training in their effects on attention network function - which may have been due to participants in the OM group practicing FA (see previous chapter for discussion of this issue). The current chapter describes a study using an integrated mindfulness-training programme, which includes features of both FA and OM training. The previous study, which used the modified (emotional) ANT did not demonstrate the predicted effects of mindfulness training on emotional processing. This may have been due to the negative word cues, presented in the modified ANT, generating insufficiently strong emotional salience in our low-trait-anxiety cohort. The study described in this chapter examined the effects of an integrated FA and OM mindfulness meditation training on attention to threat, using the anti-saccade task.

4.1.1. Threat-attention in anxiety

Anxiety is associated with distinct attentional patterns – these include reduced attentional control in the presence of negatively valenced and threatening stimuli[328], hypervigilance to threatening and negatively valenced stimuli[329], increased distractibility[328,330], delayed disengagement from threat-related stimuli, and a bias towards interpreting emotionally ambiguous stimuli as threatening[9]. The attention

control theory of anxiety describes a disruption of attentional resource-allocation balance, from 'top-down' (goal directed) towards 'bottom-up' (stimulus driven) attention[331]. These patterns may be associated with dysfunctional attention-control mechanisms underpinning pathological phenomena such as worry, anxiety and rumination. fMRI evidence lends support to the idea that anxiety is associated with reduced activity in frontal and/or prefrontal attention-regulating circuits, and with increased activation in the amygdala – resulting in heightened sensitivity to threat, coupled with impaired attention control and emotional regulation[9,82,332].

Attentional control and attentional bias in anxiety have been explored in both clinical and non-clinical populations using a range of experimental tasks. A meta-analysis of eye-tracking studies of attentional bias in anxiety[116] (N=33, n=1579) found that compared to non-anxious controls, anxious subjects demonstrated increased vigilance for threat (Hedges' g = 0.47 [95% CI: 0.25-0.69]) during free viewing and visual search, and more difficult threat-disengagement in visual search tasks only (Hedges' g = 0.54 [95% CI: 0.17-0.92]). A meta-analysis of threat-related attentional biases in anxiety (N=172, n=4031)[117] showed a consistent attentional bias across a range of experimental paradigms and conditions (Cohen's d = 0.45 [95% CI: 0.40-0.49]). Both conscious and non-conscious threat-related stimuli were associated with attentional bias. The bias was consistent across clinical diagnostic categories, and different age groups, and extended to non-clinical high anxiety subjects but not to non-anxious subjects.

As outlined previously, the spectrum of anxiety is often subdivided into state and trait anxiety. The effects of these subtypes of anxiety on attention have been proposed to be different[136] – in that state anxiety enhances the threat valence of a stimulus,

whereas trait anxiety causes attention to be directed consistently towards potential sources of threat. State anxiety is triggered by situational factors, so is likely to be associated with bottom-up processes, whereas trait anxiety is related to personality factors and therefore likely to be more associated with top-down processes[73]. There is some evidence that trait anxiety is associated with reduced executive control performance on the ANT: Pancheco-Unguetti and colleagues used a modified form of the ANT (ANT Interactions ANT-I) to test subjects with high vs. low trait anxiety scores[73]. High trait anxiety subjects demonstrated significant deficiencies in executive control network performance. State anxiety showed associations with orienting network and alerting network over-functioning. Pancheco-Unguetti and colleagues[74] compared performance on the ANT-I between patients with anxiety disorders and healthy controls. Anxiety disorders were associated with executive attentional network dysfunction and with reduced efficiency in attentional disengagement from invalid cues – including emotionally neutral cues. Furthermore, Han and colleagues showed that adolescents with comorbid depression and anxiety disorder demonstrated a faster orienting response on the ANT when compared to depressed adolescents without comorbid anxiety disorder[138].

4.1.2. Threat attention in anxiety - Experimental tasks

Attentional biases in anxiety have been demonstrated using several experimental tasks including the Stroop colour-naming task, the dot-probe task[351], and the anti-saccade task.

4.1.3. The Stroop colour-naming task

In the Stroop colour-naming task, subjects are presented with emotionally threatening and non-threatening words printed in different colours, and instructed to name the colour. Longer latency on the Stroop colour-naming task indicates interference with the primary task by the distractor (the emotional context of the word). Consistent evidence shows that clinically and non-clinically anxious subjects have longer latencies on this task when presented with threat-related words[352].

4.1.4. The dot-probe task

The dot-probe task[353] briefly presents subjects with paired pictures or words (one neutral and one emotionally valenced) stimuli in two locations on a screen. After stimulus offset, a dot probe is presented in one of the spatial locations, and the subject indicates the location in which the dot-probe appeared. Latencies are shorter when subjects are already attending to the spatial area where the dot probe is presented. Anxious subjects have shorter latencies when the dot-probe is presented in areas that previously contained threat-related words/pictures[353].

4.1.5. The Anti-saccade task

The anti-saccade task (Figure 4.1), first described by Hallett [118] has emerged as an important tool for investigating subjects' attention control (see chapter 1 for a fuller discussion of the anti-saccade task). The task requires a two-step process: 1. Suppression of the automatic pro-saccade (the tendency to look towards the target), and 2. Generating a voluntary anti-saccade towards the position mirroring the presented target. The task decouples stimulus location from saccade goal, and requires

inversion of the stimulus vector to generate the saccade vector. Anti-saccade performance can be compared to performance on a task requiring the subject to look towards the presented target (pro-saccade task), to provide information on attentional control functions of inhibition, shifting, and updating. The general patterns observed in the anti-saccade task[119] are:

1. Correct anti-saccades are generated later than correct pro-saccades;

2. Direction errors are mostly on anti-saccades, and are generated earlier than correct movements;

3. Removing the fixation marker before presenting the target is associated with reduced latency and increased anti-saccade errors;

4. Both pro-saccades and anti-saccades demonstrate bimodal distributions – a low-latency 'express saccade' (a prepotent response) in which the target stimulus is translated directly into a pro-saccade (which generates fast errors on the anti-saccade task); and a delayed saccade (volitional response) reflecting the time required for computation (which is greater for correct anti-saccades than for correct pro-saccades due to the increased complexity of the computation required).

Correct performance on the anti-saccade task requires top-down control to prevent express-saccade related directional errors. The anti-saccade task provides two performance measures[122]:

1. *Performance effectiveness* – anti-saccade accuracy rate;

2. *Performance efficiency* – correct saccade latency.

Compared to low-anxious (LA) subjects, high anxious (HA) subjects have longer correct anti-saccade (but not pro-saccade) latencies – suggesting that the anxiety-related deficit is in the inhibitory component of attentional control, leading to reduced efficiency; there were no significant differences between HA and LA subjects in error rate – investigations suggesting that anxiety reduces efficiency but not effectiveness [75,123]. Subjecting healthy participants to severe threatening-stimuli version of the anti-saccade task (using aversive images from the International Affective Picture Set) resulted in an elevated error rate (reduced effectiveness) in HA vs. LA participants[124] – this may reflect the additional cognitive processing required to over-ride the attentional bias towards threatening stimuli in HA participants. HA participants required to shift randomly between pro and anti-saccade tasks (on a mixed anti-saccade task), showed reduced ability to shift attentional resources in response to task changes, when compared to LA participants[75]. In a study of anti-saccade task under incentivised vs. non-incentivised vs. punished conditions in adolescents with GAD compared to healthy adolescents and adolescents with MDD[125], the inhibitory efficiency of the GAD group in Incentivised vs. non-incentivised trials was reduced when compared to the healthy group. A study comparing the anti-saccade task performance of adolescents with various anxiety disorders to healthy controls[126] found enhanced inhibitory control following exposure to threat cues (fear faces) only in anxiety disorder group, and following exposure to positive cues (happy faces) only in healthy controls. Use of the 7.5% CO_2 paradigm to induce anxiety in healthy individuals is associated with longer anti-saccade latencies (reduced efficiency) and with increased anti-saccade errors towards threat-related stimuli (reduced effectiveness)[127]. An fMRI study of the anti-saccade task in healthy volunteers[121] aimed to identify the activation

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pattern associated with the two main cognitive components of the task: Inhibition of the pro-saccadic reflex, and generation of the volitional anti-saccade. The study found that ventrolateral and dorsolateral prefrontal cortical areas were activated throughout both components of the task, and concluded that these areas were involved in executive task coordination. The drawback of this study was the 24-36 second duration of each trial, which may have introduced a delay in which other cognitive processes could have confounded the results. A study of evoked response potentials (ERPs) during pro-saccade and anti-saccade found that compared to LA individuals, HA individuals had longer anti-saccade latencies, and lower ERP activity, at frontocentral and central recording sites, immediately prior to correct anti-saccade trials. The authors concluded that this was evidence of anxiety-related reduced recruitment of frontal top-down attentional control resources needed for suppression of reflexive pro-saccade[128].

4.1.6. Mindfulness and attention to threat

Anxiety-related cognitive processing impairments involve executive dysfunction resulting in over-commitment of scarce attentional resources to threat-related distractors, which are task-irrelevant; giving rise to hypervigilance to threat, distractibility, and impaired disengagement from threat[24,65,117]. Mindfulness training is associated with specific effects on attention (see chapter 1 for a discussion of this area) including improved executive control[22], and would therefore be a logical candidate for assessment as an intervention for anxiety-related cognitive biases in threat processing. There are no previously reported studies of mindfulness effects on the anti-saccade task.

4.2. Aims

This study examines the effect of an integrated (FA and OM), month-long mindfulness meditation-training programme on attention to threat as measured by the anti-saccade task. In comparison to the study described in the previous chapter, the mindfulness intervention was strengthened by integration of the FA and OM modalities into one coherent programme, and by provision of an online monitoring tool to record homework practice.

We predicted that higher levels of dispositional mindfulness would be associated with improved global anti-saccade performance (reflecting a globally better inhibition performance), and improved anti-saccade performance on threat-related stimuli (reflecting better attention to threat).

We therefore predicted that the active intervention (integrated mindfulness meditation training programme) group would outperform the control group on:

- Anti-saccade performance

- Anti-saccade performance in threat-related stimuli

- Improvement in dispositional mindfulness levels (MAAS and KIMS)

4.3. Method

4.3.1. Participants

A total of 66 students from the University of Southampton, all mindfulness novices were recruited, having responded to advertised offers of course credit / money for taking part in the study. Sixteen participants were excluded due to technical problems

in capturing the baseline anti-saccade task data, and four participants were excluded following baseline testing due to extreme outlier status on the anti-saccade task (Figure 4.2). Outliers were defined using box-plots in SPSS, once all participants have completed the study. The remaining 46 participants (Mean age 20.27 years, SD = 3.08) were randomised to one of two groups: 1. Integrated mindfulness meditation training programme (n=20; M:F ratio = 0.43; mean age = 19.8 years; 2. Control group (n=26, M:F ratio = 0.44; mean age = 20.5 years).

Our sample size of 20+ participants per group provides 80% power at an alpha level of 5% to detect a medium-large effect size (Choen's d = 0.5 - 0.8), based on reviews of the effects of mindfulness on cognitive function (e.g. Chiesa et al. 2011[334]).
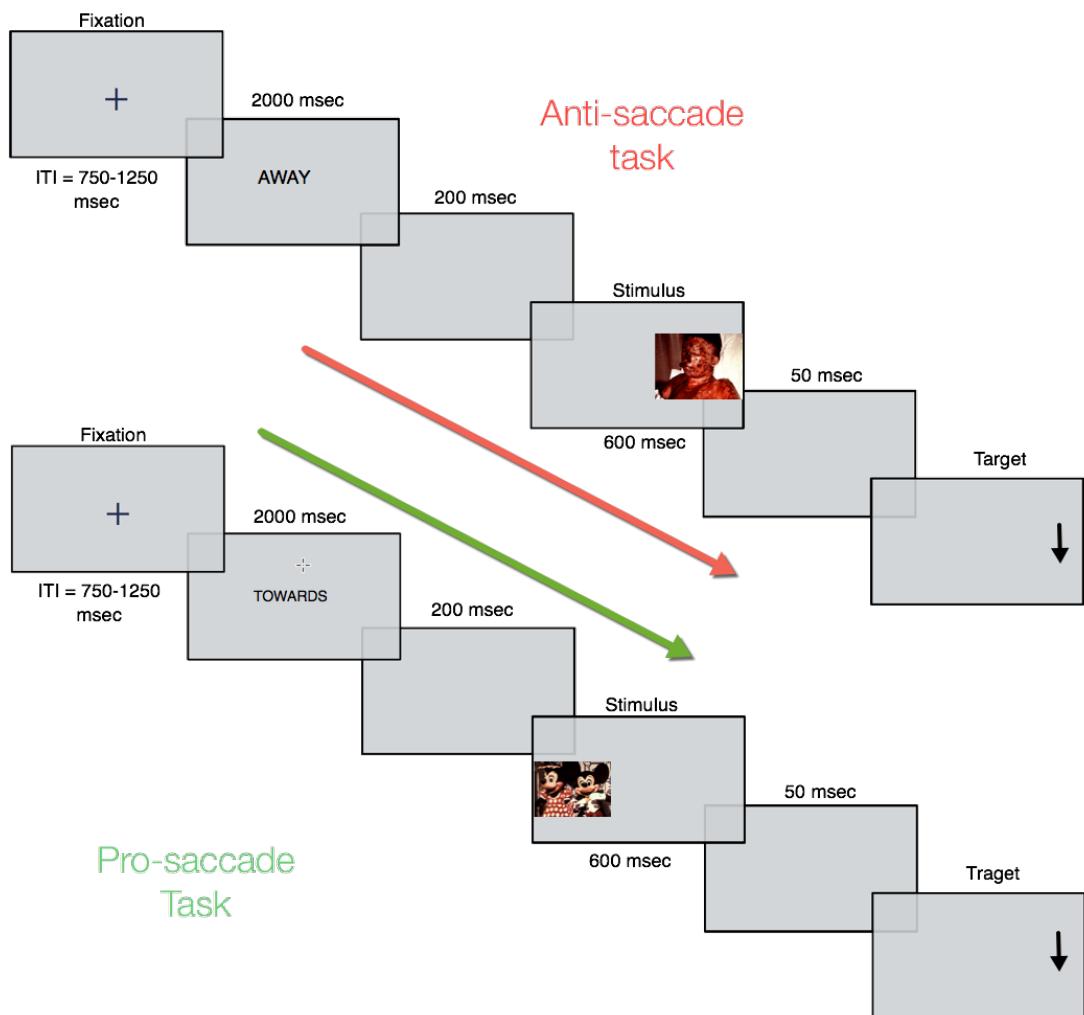
Figure 4.1 Pro-saccade and anti-saccade tasks:

Subjects fixate on a marker, and are then instructed to look either towards or away from a stimulus. ITI = Inter-trial interval (adapted from Ansari et al. 2008 and from Ainsworth 2013[129,350])

4.3.2. Study design and workflow

This was a randomised test-retest controlled study of the effects of a one-month mindfulness-training programme on threat-attention (as measured on the anti-saccade task) in healthy volunteers.

The study was approved by the University of Southampton research ethics and research governance committees. (See figure 4.2 for study workflow). Participants were recruited using advertisements offering course credits / money in exchange for study participation. Participants underwent standard briefing and informed consent was obtained. Participants completed a set of baseline rating scales (see below for details) including the state-trait anxiety inventory (STAI), attention control scale (ACS), mindful attention awareness scale (MAAS), Kentucky inventory of mindfulness skills (KIMS), Penn state worry questionnaire (PSWQ), and Spielberger state anxiety inventory (SSAI). Participants went on to complete a baseline anti-saccade task (see below). A total of 16 participants were excluded due to technical problems in capturing the baseline anti-saccade task data, and four participants were excluded following baseline testing due to extreme outlier status on the anti-saccade task (Figure 4.2). Participants were randomly assigned to either active intervention, or test-retest control groups. Training sessions were held on the Highfield campus (University of Southampton). The integrated mindfulness meditation training intervention group received six 60-minute training sessions over 5 weeks, and were also instructed to practice daily using an online audio mp3 containing a 10-minute guided meditation: the online site also kept a log of the practice record for each participant. The test-retest control group did not receive training sessions, and was not instructed to practice at home. Post-intervention assessment sessions consisted of repeated rating scales, and

another anti-saccade task. Immediately before undergoing the second anti-saccade task, participants in the active intervention group practiced mindfulness meditation for 10 minutes using the homework online audio MP3; whereas participants in the control group were instructed to sit quietly and relax for 10 minutes. There were no significant differences between the groups in the length of interval between pre- and post-intervention test-sessions [$M_{meditation}$ = 35.5, $M_{test-retest}$ = 31.1; t(54) = 1.64, p = 0.11].
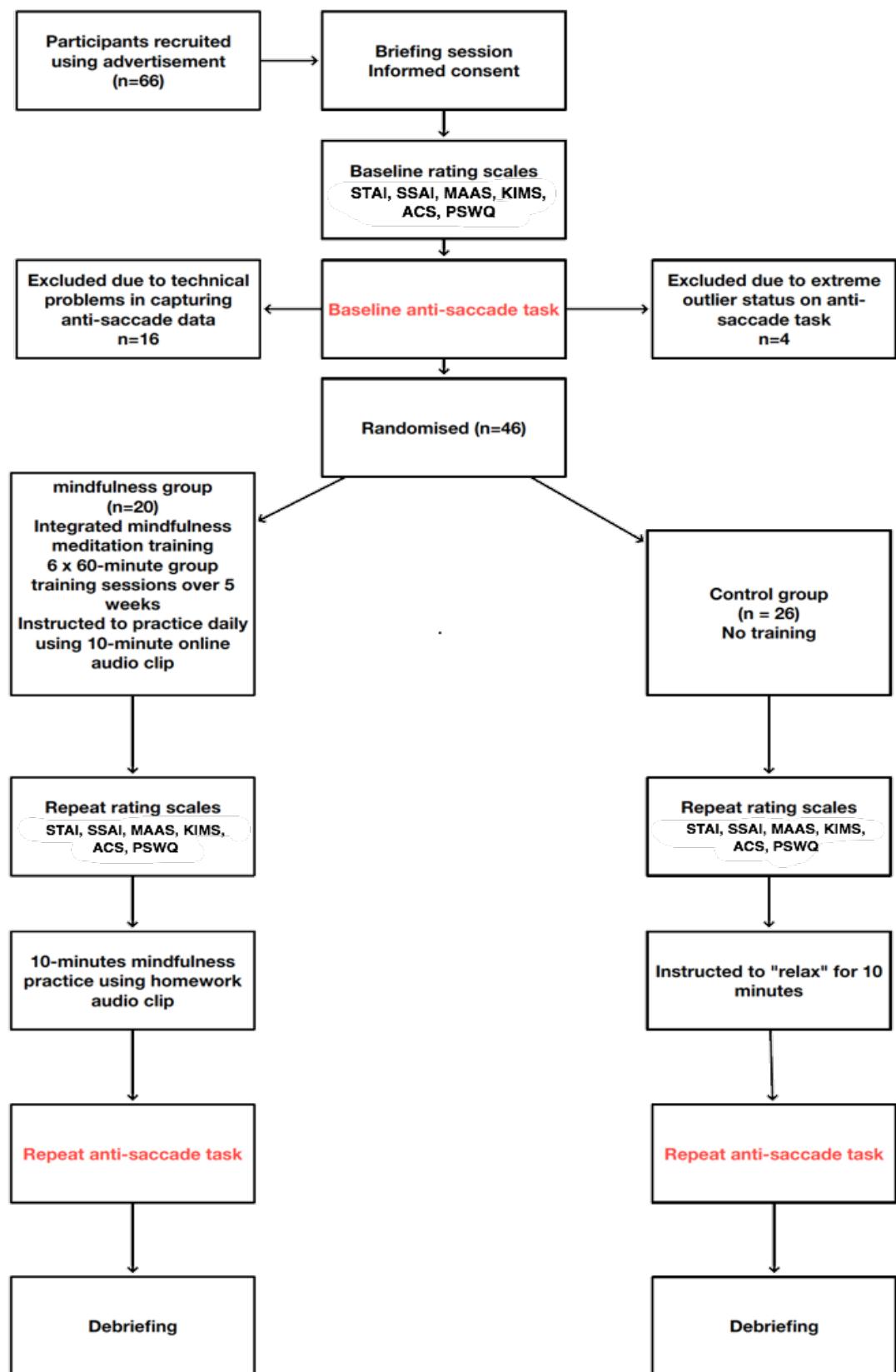
Figure 4.2: Procedural consort diagram describing Study design and workflow

4.3.3. Experimental interventions

The active intervention consisted of a 5-week mindfulness meditation-training programme. I developed and delivered the training programme based on my experience of mindfulness practice and of delivering mindfulness-based clinical interventions. I have been practicing mindfulness meditation since 1990, have accrued several thousand hours of personal practice, have attended many residential mindfulness retreats (including several retreats led by the founders of MBSR and MBCT), and have more than 10 years experience in delivering mindfulness-based clinical interventions in a variety of individual and group settings. My competence level has been assessed as level 5-6 on the mindfulness-adapted stages of competence scale[333,338]. The programme consisted of 6 sessions, each lasting about 60 minutes. The sessions were held in groups of 15-20 participants. All participants took part in all 6 sessions. Each training session included an initial introduction, followed by reflection on homework, and a number of basic mindfulness exercises. This was followed by a 20-30 minute period of guided group practice. The practice typically began with focused attention (FA) practice:

"Finding a place where the sensations of the breath are particularly clear right now…at the tip of the nose, the back of the throat, the chest or the abdomen….making a decision to stay with this place for the duration of this exercise rather than moving awareness from one place to another…turning awareness towards this place…allowing awareness to settle on this point…allowing the mind to become comfortable here…..maintaining this focus, and when the mind wanders, gently returning the mind to this place…..when the mind has wandered, lightly and firmly returning the focus to this place….really examining the sensation of the breath, and making the focus of attention as fine and as

exact as possible….really pinpoint this one point where the breath is observed."

As the session progressed, elements of open monitoring (OM) were introduced:

"Allowing a sense of awareness of the breath and physical sensations in the body generally to gradually expand….allowing awareness to include any sounds, whatever the eyes see, and perhaps any smells and tastes that may be present….allowing all of this to come within the field of awareness…sitting here, with all of this, perhaps allowing the emotional tone, how

any feelings right now, to become part of this field of awareness – whatever sense of comfort or discomfort, any emotions present, allowing that to become part of the field of awareness right now, noticing any changes that may occur…"

Participants were then encouraged to engage in a group reflective discussion. The expectation of adherence to daily homework was emphasised. Homework practice consisted of once daily individual 10-15 minute practice guided by an online audio MP3, recorded by me. The online system kept a log of each participant's homework activity, in order to capture practice time data, and also to encourage adherence to daily homework practice. At two time-points (separated by 5 minutes) during each homework sessions, participants were instructed to respond by clicking their mouse button, to confirm that they were actively participating in the practice (rather than accessing the audio and allowing it to passively run in the background). The test-retest control group did not receive any active intervention (neither group sessions nor homework).

4.3.4. Outcome measures

4.3.4.1. Primary outcome measure

4.3.4.1.1. Anti-saccade task

The stimuli used in the anti-saccade task were colour photographs taken from the International affective picture system (IAPS)[354]: Eight neutral-valence and eight negative-valence pictures were used in the study (examples in figure 4.3): normative valence ratings (Scale = -4 to +4, $M_{neutral}$ = 1.2; $M_{negative}$ = -3.1) and arousal ratings (Scale = 0-8, $M_{neutral}$ = 2.9, $M_{negative}$ = 5.8). Horizontal eye-movement measurements were collected using electro-oculography with two 8 mm pinch-electrodes placed 1cm lateral to the lateral orbital margins.
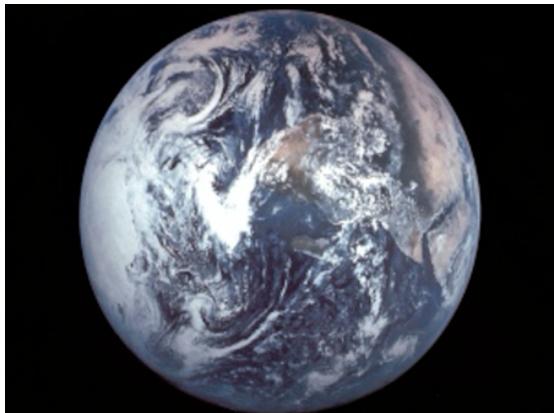
Figure 4.3: Examples of negative-valence (left) and neutral-valence pictures from the International affective picture system (IAPS)[354], used in the anti-saccade task.

Participants completed a practice block of 16 trials, followed by two blocks of 64 trials. Each block consisted of 32 pro-saccade and 32 anti-saccade trials. Trials were counter-balanced for stimulus-valence, stimulus-location, probe-location, and probe type (up/down). Each trial (figure 4.1) was separated

from the previous trial by an inter-trial interval (ITI) of 750-1250 msec (Mean = 1000 msec). A cue instructing whether the next trial was a pro-saccade or an anti-saccade was then displayed for 2000 msec, followed by a blank screen for 200 msec. The emotional stimulus was then presented for a duration of 600 msec. The target, consisting of an arrow, was presented after a blank screen inter-stimulus interval (ISI) of 50 msec. Participants were instructed to indicate whether the arrow was pointing up or down – this was done in order to increase task-demand. An assessor blinded to group assignment and to trial-type (pro/anti-saccade) used the AcqKnowledge 3.8.1 system to removed anticipatory saccades (latency < 100 msec), and aborted saccades (which did not terminate at either right or left target areas, subtended less than 6° visual angle), and then scored the remaining saccades' direction and latency.

4.3.4.2.   Secondary outcome measures:

4.3.4.2.1.   Spielberger State-Trait Anxiety Inventory[335]

The Spielberger State-Trait Anxiety Inventory is a widely accepted instrument for measuring both state and trait anxiety. It has been translated into more than thirty languages, and cited in over 3000 studies[313]. The Spielberger State-Trait Anxiety Inventory consists of 40 items, 20 aimed at assessing trait anxiety (Spielberger Trait Anxiety Inventory - STAI), and 20 measuring state anxiety (Spielberger State Anxiety Inventory - SSAI). Each item is a 4 point forced-choice Likert scale[314]. The state and trait scales each contain 2 factors: "anxiety absent" and "anxiety present". Trait anxiety is a

tendency towards feeling anxiety, worry, discomfort, and stress; it describes a relatively stable characteristic or disposition, rather than a reaction to a particular set of conditions. The STAI addresses how subjects feel generally, commonly or usually. Items on the trait scale include statements such as: "I am content", "I have disturbing thoughts". Each item on the trait scale is scored: 1 almost never; 2 sometimes; 3 often; 4 almost always. State anxiety is a transient response to a set of conditions perceived as threatening or dangerous. It can include components such as autonomic arousal, and feelings of fear, discomfort, or nervousness. The SSAI measures how subjects feel "right now, at this moment"[314]. Items on the state scale include statements such as: "I am calm", "I am worried". Each item on the state scale is scored: 1 not at all; 2 somewhat; 3 moderately so; 4 very much so. Scores generated on each scale can range from 20 – 80, higher scores reflect more intense anxiety[314]. The Spielberger State-Trait Anxiety Inventory was found to have good internal consistency (average αs > 0.89)[314]. The test-retest reliability at different time points of the trait scale (STAI) is also good (average r = 0.88)[315]. The test-retest reliability of the state scale (SSAI) at different time-points is of course lower (r = 0.70).

4.3.4.2.2.   Mindful attention awareness scale (MAAS)[336]

The MAAS is a 15-item questionnaire, inquiring about the presence or absence of attention in common everyday experiences (e.g. "I drive places on 'automatic pilot' and then wonder why I went there", and "I could be experiencing some emotion and not be conscious of it until some time later"). Each item on the MAAS invites response along a 6-point Likert scale ranging from 1: "Almost Always" to 6: "Almost Never". All items on the MAAS are constructed so that a higher score indicates a higher degree of

dispositional mindfulness, in fact all items on the MAAS are formulated as mindlessness experiences – the reason being that these were thought to be more easily accessible to non-expert meditators[339]. The MAAS has been shown to have good internal consistency (Cronbach's α in range of 0.82 - 0.87)[336].

4.3.4.2.3. Kentucky inventory of mindfulness skills [355]

The KIMS is a 39-item questionnaire used for the assessment of four mindfulness skills: Observing, describing, acting with awareness, and accepting/allowing without judgment. It includes items such as: "*I'm good at finding the words to describe my feelings*", and "*I pay attention to sensations, such as the wind in my hair or sun on my face*". Each item is rated on a 5-point Likert scale ranging from 1: "Never or very rarely true", to 5: "Almost always or always true". Some items describe the absence of a mindfulness skill (e.g. "*I believe some of my thoughts are abnormal or bad and I shouldn't think that way*", and are therefore reverse-scored. Higher scores reflect higher degree of mindfulness. The KIMS has good internal reliability (α = 0.76-0.91), and test-retest reliability (α = 0.65-0.86). The KIMS correlates positively with the MAAS.

4.3.4.2.4. The Penn State Worry Questionnaire (PSWQ) [337]

The PSWQ is a well-established self-report instrument used to measure subjects' worry trait. The PSWQ contains 16 items relating to worry; each item is scored on a 5 point Likert scale ranging from 1 "Not at all typical of me" to 5 "Very typical of me". Eleven items are positively scored – for example: "I am always worrying about something", and 5 items are scored in reverse – for example: "I do not tend to worry about things". Higher scores are correlated with increased tendency to worry. PSWQ demonstrates

good internal consistency (α = 0.90)[340], and test-retest reliability[341].

4.3.4.2.5.   Attentional Control Scale (ACS)[114]

The ACS is a 20-item self-report measure assessing the ability to maintain attentional control in the presence of distractors such as environmental stimuli, concurrent tasks, and emotional states. The ACS presents items such as "When I am reading or studying, I am easily distracted if there are people talking in the same room", and "When trying to focus my attention on something, I have difficulty blocking out distracting thoughts"; it then asks subjects to rate themselves along a 4 point Likert frequency of experience scale ranging from 1 "Almost never" to 4 "Always". The ACS demonstrates good internal consistency using a two factors Focusing (α= 0.82) and Shifting (α = 0.71) sub-scales[342].

4.3.4.2.6.   Visual analogue scales (VAS) of mood and anxiety[356]

VAS can be used to measure a construct across a continuum of values, when direct measurement is difficult or impossible to achieve. Examples of characteristics that can be measured using a VAS include subjective pain, intensity of emotional experience, or attitudinal response. It is important for the construct to be perceived as a continuum rather than a set of discrete steps or values. The VAS was presented as a 150mm horizontal line, stretching between two anchor statements defining the end points of a continuum. Participants were tasked with indicating their position along the continuum by marking the line. Measuring the distance in mm from the left end of the line to the marked point derives the score (0-150). In this study, we presented subjects with VAS scales for: 1 "anxiety" (VAS-Anx); 2 "happiness" (VAS-Happ); 3 "attentiveness" (VAS-

Att). VAS scales are widely used for measuring pain and other psychological states, and have also been validated in anxiety[357]. The evidence suggests that VAS scales perform well in comparison to subjective measures such as the Likert and Borg scales[358,359].

4.4. Results

4.4.1. Participants

Four participants were excluded from the analysis due to being extreme outliers on baseline anti-saccade task accuracy – the increased error-rate was probably due to misunderstanding of the instructions, or to inappropriate task-management methods. The mindfulness training and test-retest control groups did not significantly differ in gender (χ2 = 0.91; p = 0.57 and age [t(46) = -0.99; p = 0.26]. Groups did not significantly differ at baseline on self-report measures (Table 4.1). Groups did not differ in age (nor in gender ratio (t < 1, ps > 0.38).

Table 4.1: Mindfulness meditation vs. control group: pre- and post-intervention self-report measures.

		α	Meditation		Control		F	p
			n=22		n=24			
			M	SD	M	SD		
STAI	pre	0.89	41.3	9.0	42.6	9.3	0.02	0.63
	post	0.92	41.5	10.1	43.5	10.2	0.22	0.51
SSAI	pre	0.92	34.7	7.8	36.9	10.9	1.42	0.43
	post	0.92	36.1	10.4	37.3	9.6	0.133	0.68
MAAS	pre	0.83	53.9	11.3	54.6	11.6	0.25	0.85
	post	0.87	57.2	11.9	53.2	13.2	0.48	0.60
KIMS	pre	0.72	117.6	12.6	115.5	13.9	0.82	0.60
	post	0.77	120.0	17.5	117.4	12.7	3.97	0.65
ACS	pre	0.84	48.33	8.2	46.3	8.9	0.74	0.44
	post	0.86	49.4	7.2	46.0	8.7	1.43	0.27
PSWQ	pre	0.94	46.9	13.2	46.0	13.8	0.60	0.91
	post	0.94	45.6	12.5	45.3	11.9	0.18	0.95

STAI = Spielberger state-trait anxiety inventory; SSAI = Spielberger state anxiety inventory; MAAS = mindful attention awareness scale; KIMS = Kentucky inventory of mindfulness skills; ACS = attentional control scale; PSWQ = Penn state worry questionnaire.

4.4.2. Effects of mindfulness meditation training on attention to threat as measured on the anti-saccade task

The saccade task produces two main types of outcomes: 1. Error-rate – calculated as proportion of trials in which saccade direction was in line with the given instruction. This is a measure of effectiveness on the attention control theory (ACT); 2. Correct-saccade latency – mean saccade-latency in all correct-saccade (i.e. trials in which the saccade was in the instructed direction) trials. This is a measure of ACT efficiency, because increased latency can be associated with slower cognitive processing. Shapiro-Wilk's test confirmed that both error-rates and latencies met normality assumptions (all p's > 0.05). Saccade accuracy and latency data is summarised in table 4.2.

4.4.2.1. Saccade error rates:

A 2 (time: t1 vs. t2) x 2 (trial-type: anti- vs. pro-saccade) x 2 (emotion: negative vs. neutral) x 2 (group: mindfulness vs. control) mixed model omnibus ANOVA demonstrated the following significant effects:

- Main effect of trial-type: mean error rates on anti-saccade trials (32%) were higher than on pro-saccade trials (3%) [$F_{(1, 44)}$ = 110.77, p < 0.001].

- Trial-type x time interaction [$F_{(1, 44)}$ = 10.17, p = 0.003]: Mean anti-saccade error rates reduced from t1 (34.7%) to t2(29.3%) [$t_{(46)}$ = 2.669; p = 0.01]; mean pro-saccade error did not significantly change from t1 (2.6%) to t2 (4.2%) [$t_{(46)}$ = -1.78; p = 0.08].

A 2 (time: t1 vs. t2) x 2 (trial-type: anti- vs. pro-saccade) x 2 (emotion: negative vs.

neutral) x 2 (location: left vs. right) x 2 (group: mindfulness vs. control) mixed model ANOVA demonstrated no main effect of location on error-rates [$F(1,44) = 0.33$, $p = 0.57$], and no significant interactions of location with other variables. There was no significant effect of group, or emotion, or group by time interaction.

4.4.2.2. Correct saccade latencies:

A 2 (emotion: negative vs. neutral) x 2 (trial-type: anti- vs. pro-saccade) x 2 (time: t1 vs. t2) x 2 (group: mindfulness vs. control) mixed model omnibus ANOVA demonstrated the following significant effects:

- Main effect of time: mean correct saccade latency at t1 (M=205.07; SD = 40.57) was quicker than at t2 (M=217.94; SD = 46.32), [$F(1,43) = 4.45$, $p = 0.041$].
- Main effect of trial-type (anti- vs. pro-saccade): mean pro-saccade latency was significantly shorter than mean anti-saccade latency [$F(1,43) = 4.36$, $p = 0.043$]

There was no significant effect of group, or emotion, or group by time interaction.

4.4.3. Effects of mindfulness meditation training on self-report measures of anxiety, mindfulness and attention-control

No significant between-group differences were found on independent-sample t-test in any of the self-report measures at baseline (t1) or at t2 (table 4.1). Group-means on all self-report measures in this cohort were comparable to previously reported values in healthy volunteers[360].

Participants in the mindfulness-training group completed homework practice on an average of 27.9 days (SD = 9.4; minimum = 7; maximum = 44).

Table 4.2: Mindfulness meditation vs. control group: Saccade accuracy and latency
(msec).

				Meditation		Control	
		pro/anti-saccade	cue valence	M	SD	M	SD
Error rates	Pre	Pro	Negative	0.034	0.041	0.021	0.030
		Pro	Neutral	0.128	0.157	0.035	0.037
		Anti	Negative	0.345	0.219	0.380	0.207
		Anti	Neutral	0.322	0.189	0.339	0.178
	Post	Pro	Negative	0.030	0.297	0.048	0.095
		Pro	Neutral	0.031	0.033	0.057	0.097
		Anti	Negative	0.267	0.188	0.322	0.213
		Anti	Neutral	0.271	0.189	0.310	0.191
Latency	Pre	Pro	Negative	197.99	32.67	193.49	36.13
		Pro	Neutral	200.38	31.26	196.65	33.25
		Anti	Negative	223.54	84.95	198.66	56.53
		Anti	Neutral	226.51	79.05	204.61	49.77
	Post	Pro	Negative	219.15	45.68	198.76	29.84
		Pro	Neutral	219.87	47.89	202.27	34.55
		Anti	Negative	245.55	82.41	208.21	59.42
		Anti	Neutral	247.12	79.43	205.20	63.62

4.4.4. Adverse outcomes and side effects

No adverse outcomes or side effects were reported during this study.

4.5. Discussion

4.5.1. Attention to threat as measured by the anti-saccade task:

Saccade accuracy reflects task effectiveness as described in the ACT model. The significant main effect of saccade type, whereby the accuracy in pro-saccade trials was significantly higher than in anti-saccade trials, is an inherent feature of the anti-saccade task: correct pro-saccades are reflexive, whereas correct anti-saccades require an intentional over-ride of the pro-saccadic reflex, and initiation of anti-saccade; these involve additional cognitive processing, and are associated with increased error-rates. The second significant finding was an interaction between saccade-type and time: the accuracy in anti-saccade trials improved from t_1 to t_2, whereas pro-saccade accuracy did not significantly change. This is likely to reflect a ceiling effect in pro-saccade accuracy, which were above 95% to begin with. The improved accuracy in anti-saccade trials is likely to reflect non-specific effects (learning, and reduced stress levels at t_2), rather than intervention-specific effects. The lack of other significant effects in terms of saccade accuracy accords with the ACT model, which predicts that task effectiveness, is not affected by changes in anxiety because of compensatory mechanisms, which reduce efficiency but preserve effectiveness.

Correct-saccade latency is a measure of efficiency as defined in the ACT model, reflecting the time-cost of additional cognitive processing recruited in order to compensate for attentional inefficiencies. The main effect of time, whereby mean

correct saccade latency at t_2 was higher than at t_1 is seemingly counter-intuitive, because one might expect that the task would become more familiar and therefore less demanding. However, if one considers that the accuracy rates in anti-saccade trials improved from t_1 to t_2, then it may be that this improvement in effectiveness is achieved at the cost of reduced efficiency (as predicted by ACT). The reason that this compensation did not already occur at t_1, may be that some of the change from t_1 to t_2 is not an anxiety-related process, which can be compensated for by attentional control, but is instead a task-learning effect which is not amenable to the kind of compensation described by the ACT model. The lack of significant differences between the mindfulness and control groups may be related to the low trait-anxiety of the cohort, coupled with the non-anxiogenic nature of the task and of the surrounding conditions – in effect, participants' anxiety levels were not high enough to allow a potential treatment for anxiety to demonstrate its effect, compared to a control group. This explanation would be in line with the lack of significant effects found on self-report measures of trait and state anxiety, worry and attention control. An alternative explanation for the lack of separation between the mindfulness and control groups in the anti-saccade task, is that the threat in this study evoked a 'strong-situation'[361] whereby regardless of dysfunctional-anxiety, a functional fear response was elicited, which is not amenable to change in the same way that dysfunctional anxiety would be. It may of course be that mindfulness simply lacks effect under these conditions.

4.5.2. Self-report measures of anxiety, mindfulness and attention-control

This study did not find any significant effects of mindfulness training on any of the self-report measures of state-anxiety, trait-anxiety, attention-control, or worry. This may be due to the low-anxiety cohort of healthy volunteers recruited to this study.

This study did not find significant effects of mindfulness training on dispositional mindfulness as measured by the MAAS or the KIMS. This finding could be due to the mindfulness intervention being ineffective in improving dispositional mindfulness; or the intervention may have improved mindfulness in a way that is not being picked up by the MAAS or the KIMS. Using self-report measures to assess mindfulness is a controversial area[362] (see Grossman (2011) for a detailed discussion)[363], and the MAAS in particular has been criticised for being too focused on the awareness component of mindfulness, and for neglecting the acceptance component, which has been shown to be key to mediating the relationship between mindfulness and anxiety[364]

4.6. Limitations

There were several limitations to this study – (these are further discussed in section 8.5.2):

The lack of an active control group – the control group received no training, whereas the active intervention group received 6 hours of face to face training, as well as daily homework practice.

The negatively valenced cues used in the antisaccade task may not have been sufficiently disturbing to elicit enough anxiety in this low-anxiety cohort

The mindfulness training intervention was not associated with significant effects on participants' levels of dispositional mindfulness as measured using the MAAS and KIMS.

4.7. Implications and future prospects

This study did not demonstrate significant effects of the mindfulness training vs. control groups on threat processing or on self-report measures of anxiety, attention-control, worry, or mindfulness. This may have been due to the combination of a low-trait-anxiety cohort, with a task that did not place sufficient cognitive demands on participants in non-anxiety provoking conditions. In order to test the mindfulness intervention, it is necessary to increase the levels of anxiety in which the anti-saccade task is being performed. The next chapter describes a study in which healthy volunteers undertake the anti-saccade task while exposed to an anxiety-provoking environment in the form of breathing air enriched with 7.5% CO_2.

4.8. Funding

5. CHAPTER 5: THE EFFECTS OF A SINGLE SESSION OF MINDFULNESS MEDITATION ON ATTENTION CONTROL IN THE 7.5% CO$_2$ CHALLENGE - A NOVEL EXPERIMENTAL HUMAN MODEL OF ANXIETY

5.1. Introduction

The previous chapter described a study comparing the effects of a course of integrated (FA & OM) mindfulness training vs. test-retest control, on attention control in healthy volunteers, as measured on the emotional anti-saccade task. The study did not find significant between-group differences on any outcome measures. This may have been due to the low anxiety conditions in which the anti-saccade task was undertaken. It is also possible that the mindfulness intervention was not sufficiently powerful to demonstrate an effect in a low-anxiety group. The current chapter describes a study aiming to address those potential limitations in two ways: first, increasing the level of anxiety under which the participants undertake the anti-saccade task, by using the 7.5% CO$_2$ challenge; and second, strengthening the mindfulness intervention, by delivering a single guided session of mindfulness within the testing session itself, in order to increase the likelihood of the participants experiencing a mindful state (unlike the previous study which administered a course of mindfulness training aimed at setting up a mindfulness-trait).

5.1.1. The 7.5% CO$_2$ challenge

As described in more detail in chapter 1, inhalation of air containing elevated concentrations of carbon dioxide (CO$_2$) has long been known to induce symptoms of anxiety and panic[144], and has been used to this effect in healthy subjects[145,146].

Inhalation of concentrations of CO_2 greater than 7.5% is associated with a range of subjective and objectives manifestations of anxiety. Physiologically, there are signs of autonomic arousal including elevated blood pressure, pulse rate and sweating. Healthy subjects describe feelings of anxiety, tension and fear[149-151]. The effects of 7.5% CO_2 challenge are less pronounced than the panic symptoms associated with inhaling 35% CO_2[365]. Section 1.2.1 provides a detailed description of the evidence supporting the 7.5% CO_2 challenge as a potential novel experimental model of subjective, autonomic and neuropsychological features of generalized anxiety in humans that can be useful in the early-phase evaluation of therapeutic interventions for GAD.

5.1.2. Attention to threat in anxiety

Anxiety is associated with distinct attentional patterns – these include reduced attentional control in the presence of negatively valenced and threatening stimuli[328], hypervigilance to threatening and negatively valenced stimuli[329], increased distractibility[328,330], delayed disengagement from threat-related stimuli, and a bias towards interpreting emotionally ambiguous stimuli as threatening[9]. The attention control theory of anxiety describes a disruption of attentional resource-allocation balance, from 'top-down' (goal directed) towards 'bottom-up' (stimulus driven) attention[331]. These patterns may be associated with dysfunctional attention-control mechanisms underpinning pathological phenomena such as worry, anxiety and rumination. Section 5.1.2 provides a more detailed discussion of this area.

5.1.3. Mindfulness

Mindfulness-based interventions are a potentially useful treatment modality for anxiety disorders. Current evidence-based treatment guidelines recommend CBT as the first-line psychological intervention in mild-moderate anxiety[12]; however, CBT can be costly, difficult to access, and may be sub-optimally acceptable to patients who wish to practice at home. There is therefore a need for alternative psychological treatments for mild-moderate anxiety, that can be delivered in group-settings, at lower costs, and practiced at home with remote/online support[366]. Two main modalities of mindfulness-based interventions are described in the literature: Mindfulness Based Stress Reduction (MBSR), and Mindfulness Based Cognitive Therapy (MBCT). The core feature of mindfulness as practiced in the context of therapeutic interventions is the development of a particular kind of attentional quality – practitioners are trained to pay attention *'on purpose, in the present moment and non-judgmentally'*[172](see chapter 1 for a detailed discussion of mindfulness).

5.1.3.1. Mindfulness and attention to threat

Anxiety-related cognitive processing impairments involve executive dysfunction resulting in over-commitment of scarce attentional resources to threat-related distractors, which are task-irrelevant; giving rise to hypervigilance to threat, distractibility, and impaired disengagement from threat[24,65,117]. Mindfulness training is associated with specific effects on attention (see chapter 1 for a discussion of this area) including improved executive control[22], and would therefore be a logical candidate for assessment as an intervention for anxiety-related cognitive biases in threat processing. There are no published studies of mindfulness effects on the anti-saccade task.

5.1.3.2. Mindfulness effects on anxiety

The evidence base for efficacy of mindfulness-based interventions in anxiety is developing. A recent overview of 5 systematic reviews and meta-analyses[189], (n=2525) demonstrated that compared to waiting list or treatment as usual controls, MBCT and MBSR significantly improved symptoms of anxiety (Cohen's d = 0.49 [95% CI: 0.37-0.61]). A meta-analysis of trans-diagnostic interventions for anxiety demonstrated a significant effect of mindfulness/acceptance interventions (N=6 RCTs) on anxiety measures (Hedges' *g* = 0.61 [95% CI 0.37–0.86])[187]. A recent meta-analysis of treatments for anxiety[195] reported that the pre-post effect size for mindfulness interventions in anxiety (N=4 RCTs) was numerically the highest compared with all psychotherapies (Cohen's *d* = 1.56 [95% CI: 1.20–1.92]). A meta-analysis of MBSR in healthy subjects[196] reported a significant pre-post effect on anxiety measures (8 RCTs) (Hedges' g = 0.55 [95% CI: 0.19 - 0.92] p < 0.005). (See chapter 1 for a detailed account of the evidence base of mindfulness in anxiety).

5.1.3.3. Mindfulness effects on cognitive and emotional processing

Mindfulness training is directly concerned with developing attentional skills, as well as enhancing acceptance of emotional states. The ability to maintain attentional focus and emotional equilibrium, while accepting internal and external stimuli is a core feature of mindfulness training [207]. It is therefore not surprising that effects of mindfulness on measures of attention, cognition, and emotional processing have been found following both brief and intensive mindfulness-based interventions: A course of MBSR was associated with attenuated physiological responses to stress,

including reductions of blood pressure[367,368], and salivary cortisol[368,369]. Less structured mindfulness interventions have been shown to reduce heart-rate in healthy subjects[370]. Mindfulness-meditation is associated with improved cognitive and emotional processing in non-clinical populations[334] including key processes implicated in anxiety disorders: Experienced meditators showed enhanced executive control and orienting performance on the ANT compared to non-meditators[208,209]. A short course of meditation training was associated with improved executive control performance[210], and orienting[208] network performance on the ANT. Experienced meditators showed improved alerting network performance on the ANT following attendance of a meditation retreat[208]. Brief mindfulness training was associated with improved performance on an N-back task[220]. Intensive mindfulness retreat training (1-3 months) was found to be associated with improved performance on dichotic listening tasks[221,222], with improved detection of target stimuli post distraction, and with reduced

distractor attention resource allocation[223]. Improved working memory, sustained attention and performance on a switching task were associated with novice meditators post attendance at a 10 day meditation retreat[224].

However, current understanding of the mechanisms of action underlying mindfulness effects on anxiety, cognition, and emotional processing, is limited. There are several potential gains to be made by clarifying the mechanisms by which mindfulness-based interventions bring about improvement in anxiety: First, understanding the mechanisms may facilitate the development of more streamlined approaches, doing away with 'non-essential' intervention components, potentially shortening and simplifying the intervention. Second, clearly identifying the 'active ingredients' may

allow the development of more potent mindfulness-based interventions. And Third, understanding the mechanisms by which mindfulness-based interventions work in anxiety may enable transfer of some of these mechanisms in order to enrich/strengthen existing non-mindfulness-based therapeutic approaches, and the development of novel treatments for anxiety. The current study is the first to utilise a human experimental model of anxiety to evaluate and compare two core psychological components of mindfulness meditation: namely focussed attention (FA) and open monitoring (OM).

5.1.3.4. Mindfulness – focused attention (FA) and open monitoring (OM)

Theoretical models of mindfulness (see chapter 1 for detailed discussion) highlight the role of attention training as a core component of mindfulness interventions: Attention is one of Shapiro's three mindfulness axioms[200]; and Holzel describes "attention regulation" as a key mindfulness component[207]. Mindfulness-based interventions can be divided into two broad attentional training categories, each can be seen as a neuropsychologically distinct component: focused attention (FA), and open monitoring (OM)[316-319].

5.1.3.4.1. Focused attention (FA) training

FA practices[316] emphasise sustaining selective attention on a selected object of meditation. The meditator is instructed to concentrate their attention on the chosen object, to monitor their awareness, and when their attention wanders away from the object, to return the focus of attention back to this object. FA training commonly utilises narrow focused objects such as the sensation of the breath in a particular part

of the body. The skills/functions practiced in FA training are[317]: 1. maintaining prolonged, stable attention on a chosen object; 2. monitoring for distraction without destabilising attentional focus on the primary object; 3. disengaging from distractions; 4. redirecting attention to the primary object. These functions have been linked to neural networks involved in conflict monitoring (including DLPFC and dorsal ACC)[320-322], selective attention (Including temporoparietal junction, ventrolateral PFC, and FEF)[69], and sustained attention (including thalamus and right frontal and parietal regions)[323,324]. As FA skills develop, the practice becomes increasingly effortless, and gradually induces stable (trait) improvements in attentional control, whereby attention focuses, and rests more readily on any selected object[316].

5.1.3.4.2. Open monitoring (OM) training:

OM practices[316] commonly begin by stabilising attention on a narrow focus, and then expand the focus to include whatever comes up in the field of awareness from moment to moment. OM training develops the following skills: 1. attention devoid of particular focus on a primary object; 2. non-reactive monitoring. 3. non-reactive awareness of automatic cognitive / emotional elaborations of sensory stimuli. OM practice is characterised by an accepting, inclusive openness towards any sensory, affective, or cognitive experience presenting within the field of awareness[317,318]. Buddhist psychology regards all of these experiences as sensory experiences, as the ability to sense emotions, thoughts and images is regarded as another sensory modality[325]. There is evidence that OM practice is associated with Improved conflict monitoring[210], reduced attentional blink, improved efficiency of resource allocation to targets presented serially[326], and more distributed attentional focus[327]. As the practice

matures, it becomes increasingly effortless, and a 'non-grasping' trait develops alongside a less reactive, more sensitive awareness of one's internal and external environment.

5.1.4. The Anti-saccade task

The anti-saccade task (Figure 4.1), first described by Hallett [118] has emerged as an important tool for investigating subjects' attention control (see chapter 1 and chapter 4 for a fuller discussion of the anti-saccade task). The task requires a two-step process: 1. suppression of the automatic pro-saccade (the tendency to look towards the target), and 2. generating a voluntary anti-saccade towards the position mirroring the presented target (i.e. opposite side of the screen). The task decouples stimulus location from saccade goal, and requires inversion of the stimulus vector to generate the saccade vector. Correct performance on the anti-saccade task requires top-down control to prevent express-saccade related directional errors. The anti-saccade task provides two performance measures[122]:

1. *Performance effectiveness* – anti-saccade accuracy rate;

2. *Performance efficiency* – correct saccade latency.

5.1.5. Aims and predictions

This study compares the effects of two types of meditation training (FA and OM) vs. a relaxation control intervention, on attention-control in meditation-naive healthy volunteers, using performance on the anti-saccade task as the primary outcome measure. Participants undertook one session of guided mindfulness meditation

immediately before starting a 20 minute 7.5% CO_2 challenge, in the midst of which they completed a modified (emotional) anti-saccade task. We used the modified (emotional) anti-saccade in order to investigate the effects of mindfulness training on neutral/negative picture cues.

We predicted[371]:

- That compared to the control group, both FA and OM practice would be associated with reduced self-report anxiety, autonomic arousal, and attention to threat during CO_2-challenge.

- That OM practice would be associated with greater effect on subjective affective experience of the session, whereas FA practice would have a greater effect on attention control during the session.

5.2. Method

5.2.1. Participants

32 students (mean age = 21.7 years, SD = 3.2) from the University of Southampton (22 women and 10 men, all mindfulness novices) were recruited, having responded to advertised offers of course credit / money (£20) for taking part in the study. Potential participants were screened using the Mini International Neuropsychiatric Interview (MINI), a structured, DSM-IV-based diagnostic interview[372]. Exclusion criteria included: Pregnancy, history of respiratory and/or cardiovascular disorders, hypertension (>140 systolic and/or 90 diastolic), or migraine; use of medication in previous 8 weeks (except for topical treatments; occasional aspirin or paracetamol; oral, injectable or skin patch contraception); experience in mindfulness meditation, current or lifetime history of

psychiatric illness (including lifetime history/family history of panic attacks), regular smoker (more than 6 cigarettes/day), body mass index < 18 or > 28 kg/m2, current or past drug or alcohol dependence and recent use of illicit drugs (during previous 8 weeks) or alcohol (verified by breath test). Participants were randomised to one of three experimental groups in a single-blind, between-group design (figure 5.1):

- Focused attention (FA): n = 11, mean age = 20.5 years, M:F ratio = 0.57.

- Open monitoring (OM): n = 11, mean age = 20.5 years, M:F ratio = 0.38.

- Relaxation control (RC): n = 10, mean age = 24.0 years, M:F ratio = 0.43.

Our reported sample size of 10 + participants per group provides 75% power at an alpha level of 5% to detect a medium-large effect size (Hedge's g = 0.63, effects of MBSR on anxiety, Hofmann et al. 2010[373]).
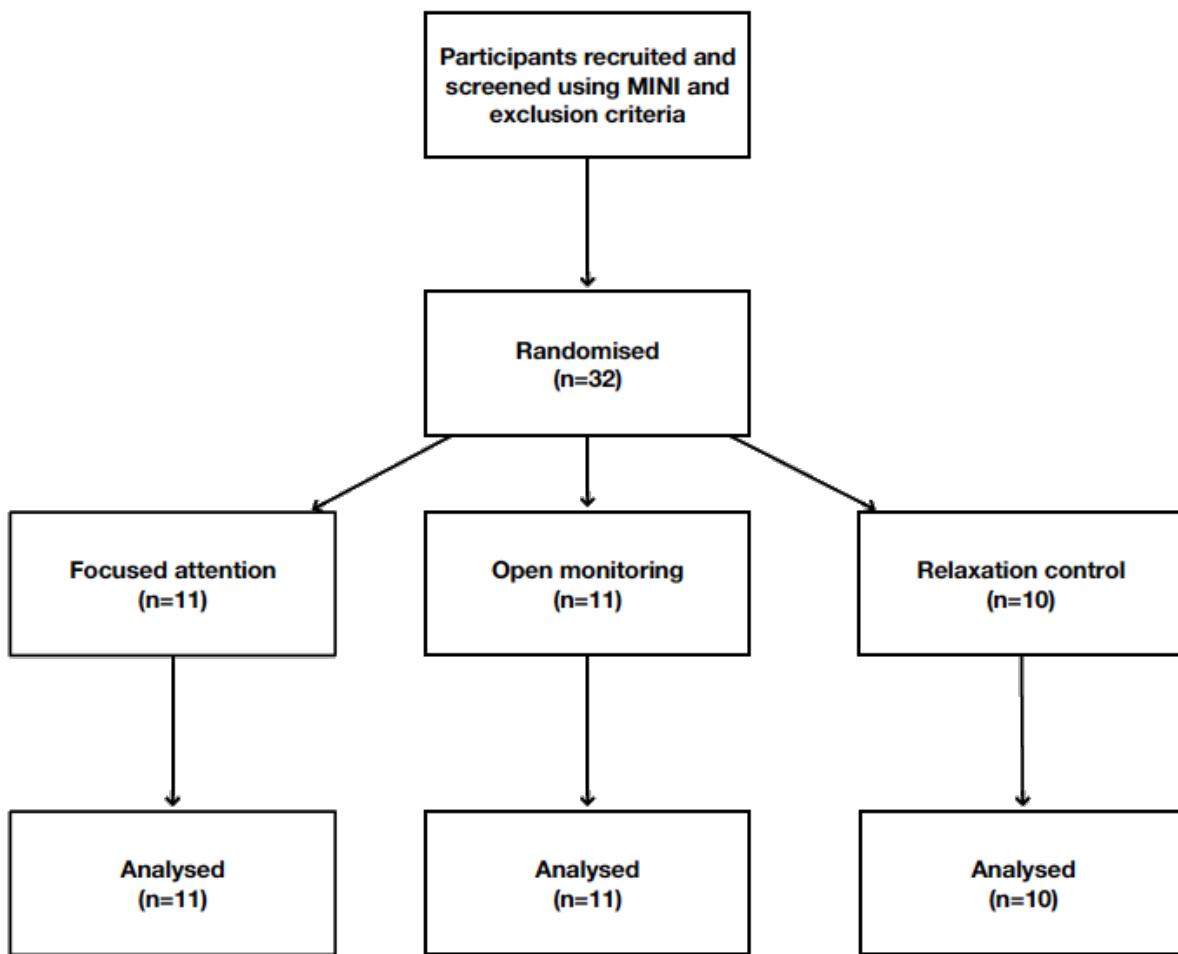
Figure 5.1: Consort diagram showing study design and progress of subjects through the study.

5.2.2. Study design and workflow

The study was approved by the University of Southampton research ethics and research governance committee (See figure 5.2 for study workflow). Participants attended a single session, underwent standard briefing, and informed consent was provided. Participants completed a set of baseline rating scales (see below for details) including the state-trait anxiety inventory (STAI), attention control scale (ACS), and the mindful attention awareness scale (MAAS). A set of outcome measures were taken at baseline, after the mindfulness/control intervention, and after the 7.5% CO_2 challenge:

heart rate, diastolic and systolic blood pressure (using Omron-M6 arm-cuff monitor, Medisave, UK), and visual analogue rating scales (from 0: Not al all to 100: Extremely) for 'anxious', 'nervous', and 'worried'. Participants also completed the Spielberger state anxiety inventory (SSAI)[8] and the Positive and Negative Affect Scale (PANAS)[374] at baseline, and immediately after the 7.5% CO_2 Challenge.
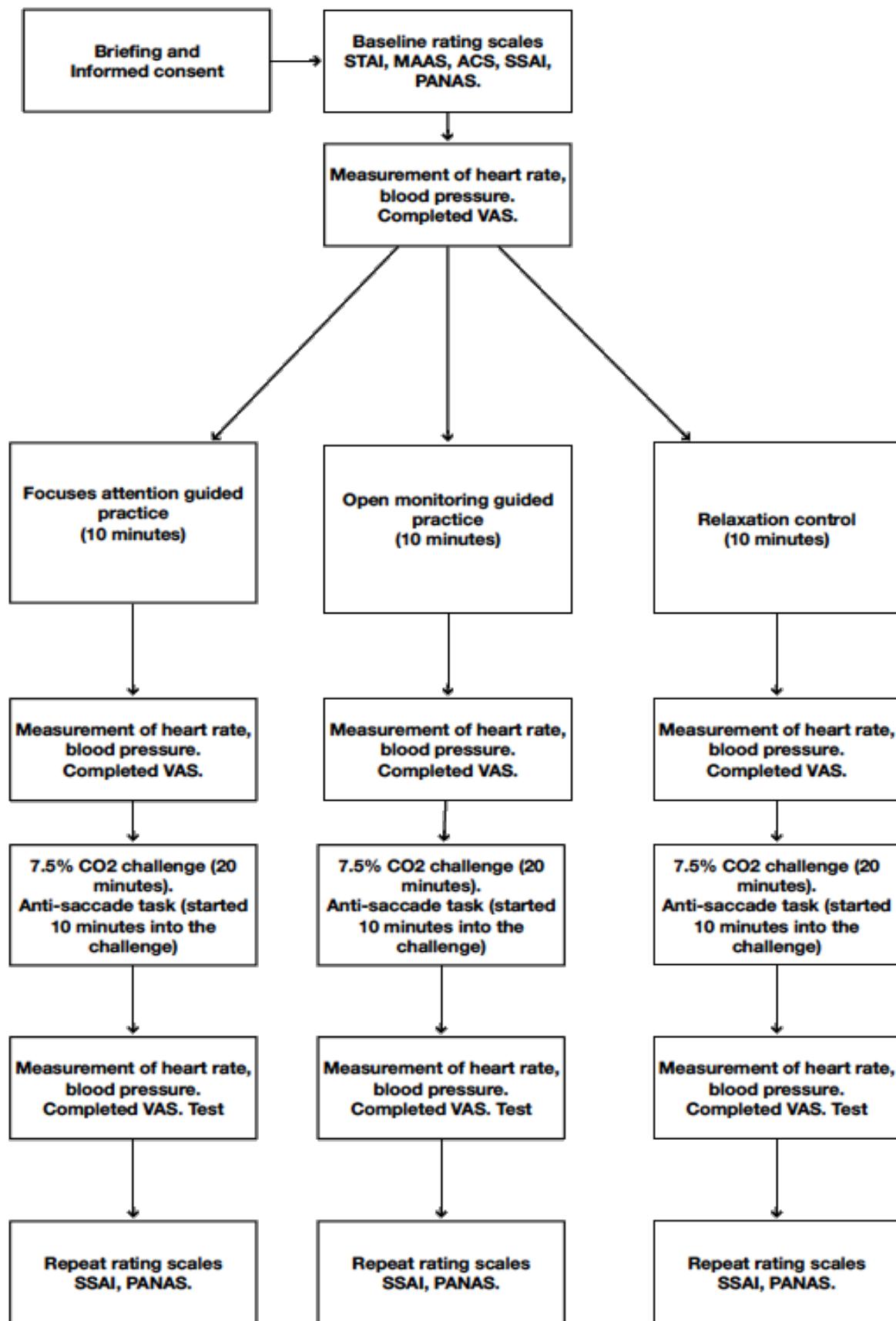
Figure 5.2: Procedural diagram describing Study design and workflow

5.2.3. Experimental interventions

The mindfulness interventions in this study took the form of a single 10-minute session of guided meditation, which was delivered immediately prior to commencing the 7.5% CO_2 challenge. It would have been technically complicated to deliver the mindfulness intervention concurrently with the 7.5% CO_2. Delivering the mindfulness intervention at the same time as undertaking the antisaccade task would have interfered with performance the antisaccade task. The guided meditation audio tracks were identical to the homework-guided meditation that was used in the study described in chapter 3. Participants assigned to the relaxation control group were instructed to relax for 10 minutes. Control group participants were instructed to relax for 10 minutes.

5.2.3.1. Focused attention (FA) training

The FA group listened to a 10-minute guided FA meditation, emphasising the development of one-pointed concentration on the sensations of breathing:

"…Finding a place where the sensations of the breath are particularly clear right now…at the tip of the nose, the back of the throat, the chest or the abdomen…. making a decision to stay with this place for the duration of this exercise rather than moving awareness from one place to another…turning awareness towards this place…allowing awareness to settle on this point…allowing the mind to become comfortable here…maintaining this focus, and when the mind wanders, gently returning the mind to this place…..when the mind has wandered, lightly and firmly returning the focus to this place….really examining the sensation of the breath, and making the focus of attention

as fine and as exact as possible….really pinpoint this one point where the breath is observed…"

5.2.3.2.   Open monitoring (OM) training

The OM group listened to a 10-minute guided OM meditation; participants were encouraged to briefly stabilise their awareness by using a short focused awareness stage, but rather than staying with focus, they were instructed to open the focus of awareness:

"…Allowing a sense of awareness of the breath and physical sensations in the body generally to gradually expand…. allowing awareness to include any sounds, whatever the eyes see, and perhaps any smells and tastes that may be present…. allowing all of this to come within the field of awareness…sitting here, with all of this, perhaps allowing the emotional tone, how
Any feelings right now, to become part of this field of awareness – whatever sense of comfort or discomfort, any emotions present, allowing that to become part of the field of awareness right now, noticing any changes that may occur…"

5.2.3.3.   7.5% CO_2 challenge

Participants used an oro-nasal face mask to breath a mixture of: CO_2 7.5%, O_2 21%, and N_2 71.5%. The session lasted 20 minutes. 10 minutes into the session, participants undertook a modified (emotional) anti-saccade task (see section 5.2.1.1. below).

5.2.4.   Outcome measures

5.2.4.1. Primary outcome measure

5.2.4.1.1. Anti-saccade task:

This study used the modified (emotional) version of the anti-saccade eye-movement task (see Chapter 1 and chapter 4 for a more detailed discussion of the anti-saccade task). The emotional cues used in the task were 8 negative and 8 neutral pictures selected from the standardized International Affective Picture Set[143,354]. Participants completed 96 randomly-ordered trials (24 trials per saccade-type x picture valence condition). Trials were counter-balanced for stimulus location. The task was presented using Inquisit 2 computer software. Horizontal eye-movements were measured by electro-oculography and sampled at 1000 Hz (MP150-amplifier and AcqKnowledge 3.8.1 software, Biopac systems, Goleta, CA). Saccade-data was pre-analysed similarly to the methodology described in Chapter 4. The data was scored by a blinded assessor.

5.2.4.2. Secondary outcome measures:

5.2.4.2.1. Spielberger State-Trait Anxiety Inventory[335]

The Spielberger State-Trait Anxiety Inventory is a widely accepted instrument for measuring both state and trait anxiety. Detailed discussion of the Spielberger State-Trait Anxiety Inventory is provided in section 4.3.4.2.1.

5.2.4.2.2. Mindful attention awareness scale (MAAS)[336]

The MAAS is a 15-item questionnaire, inquiring about the presence or absence of attention in common everyday experiences . Detailed discussion of the MAAS is

provided in section 4.3.4.2.2.

5.2.4.2.3. The five facet mindfulness questionnaire (FFMQ)[375]

The FFMQ is a 39-item self-report measure of mindfulness; it scores each item on a five-point Likert scale. The FFMQ consists of five dispositional mindfulness subscales: observing, describing, acting with awareness, non-judgment of inner experience and non-reactivity to inner experience. Scores on subscales range from 8-40, with higher scores reflecting

Increased mindfulness. This study used the FFMQ instead of the KIMS, as (unlike the KIMS), it is not constructed around the concepts of dialectical behaviour therapy (DBT).

5.2.4.2.4. The Positive and Negative Affect Schedule (PANAS)[374]

The PANAS is a widely used, 20-item, 5-point Likert scale measure of current positive and negative affect. Subjects are asked to report to what extent they presently experience a range of emotions (e.g. "Proud", "Active", "Nervous", "Ashamed") from 1: "very slightly or not at all" to 5: "extremely". Scores range from 10-50, higher scores indicating increased positive affect and reduced negative affect. The PANAS was used as an outcome measure to assess affective state in addition to Visual Analogue Scales, as the VAS did not capture significant differences between groups in the previous study. Two 10-item sub-scales (PANAS-P and PANAS-N) measure positive and negative affect independently, with good internal consistency, PANAS-P α = .89, PANAS-N α = .85[376].

5.2.4.2.5. Attentional Control Scale (ACS)[114]

5. The ACS is a 20-item self-report measure assessing the ability to maintain attentional control in the presence of distractors such as environmental stimuli,

concurrent tasks, and emotional states. Detailed discussion of the ACS is provided in section 4.3.4.2.5.

Visual analogue scales (VAS)

visual analogue ratings quantified the extent to which participants felt 'anxious', 'nervous', and 'worried' along a response scale ranging from 'Not at all' (0) to 'Extremely' (100). Visual analogue ratings were averaged to provide a composite anxiety score[371]

5.3. Results

5.3.1. Participants

Participants were randomised to 3 groups :

A Freeman-Halton extension of Fisher's exact test confirmed that the FA, OM, and control groups did not significantly differ in gender (p = 0.89) or age (p = 0.20) [F=3.95; p = 0.04). One-way ANOVAs confirmed that there were no statistically significant differences between groups in their baseline self-reported measures of dispositional mindfulness (as measured on the MAAS and FFMQ), trait-anxiety (STAI), or attention control (ACS)(see table 5.1). Levels of anxiety in the cohort were in keeping with expected levels in healthy volunteers[343]; dispositional mindfulness levels on the MAAS were in keeping with normative values for young adults[344]. All self-report measures demonstrated good internal consistency (a's > 0.74).

Table 5.1: Focused attention (FA), open monitoring (OM) and control group: baseline (pre-intervention) self-report measures.

	FA		OM		Control		F(2,29)	p
	n=11		n=11		n=10			
	M	SD	M	SD	M	SD		
STAI	33.4	6.5	35.0	5.4	33.4	5.0	0.27	0.77
MAAS	61.8	6.6	55.8	6.9	57.9	6.5	2.26	0.12
ACS	49.2	8.3	52.4	5.7	50.7	8.5	0.46	0.63
FFMQ	128.1	17.5	128.6	9.2	131.2	14.6	0.14	0.87

STAI = Spielberger state-trait anxiety inventory; SSAI = Spielberger state anxiety inventory; MAAS = mindful attention awareness scale; FFMQ = five facet mindfulness questionnaire; ACS = attentional control scale; PSWQ = Penn state worry questionnaire

5.3.2. Effects of focused attention (FA) and open monitoring (OM) on attention control as measured on the anti-saccade task during 7.5% CO_2 challenge

Anti-saccade error rates and latencies were entered into separate mixed-design ANOVAs with cue-valence (negative vs. neutral) and groups (FA vs. OM vs. Control) as independent variables. Saccade accuracy rate was significantly lower on anti-saccade trials with neutral vs. negative cues [$F_{(1,28)} = 10.21$; $p < 0.03$]. An omnibus test did not find any significant main effects of group, or interactions with group (All F's < 1.72; All p's > 0.197).

Table 5.2: Group X cue-valence ANOVA of mean error rates and correct saccade latency.

Group	Focused attention (FA)		Open monitoring (OM)		Control		F(4,58)	*p*
	n=11		n=11		n=10			
Cue-valence	Neg	Neut	Neg	Neut	Neg	Neut		
% error-rate (SD)	56 (22)	61 (22)	56 (23)	59 (22)	42 (17)	*56* *(22)*	*1.17*	*0.33*
Correct saccade latency (SD)	183.1 (45.6)	182.4 (57.8)	168.4 (23.0)	190.8 (39.5)	178.7 (20.4)	*183.4* *(40.2)*	*1.29*	*0.29*

5.3.3. Effects of focused attention (FA) and open monitoring (OM) on self-report anxiety

A mixed-design ANOVA of the averaged VAS composite anxiety score was undertaken, with Group (FA, OM, RC) as between-subjects factor, and Time (baseline, post-intervention, post-CO_2 challenge) as a within-subjects factor; this found a significant interaction of Group x Time [$F_{(4,58)}$ = 3.19; p = 0.020; $\eta2P$ = 0.18] (see Figure 5.3). All three groups demonstrated a reduction in anxiety levels from baseline to the post-intervention time point (all p's ≤ 0.05), but there was no significant between-groups difference in the size of this reduction [$F_{(2,29)}$ = 0.69; p = 0.51]. There was however a significant between-group difference in the effect of 7.5% CO_2 inhalation on levels of reported anxiety [$F_{(2,29)}$ = 4.42; p = 0.021, $\eta2P$ = 0.23]: The OM group reported non-significant increase in anxiety following the 7.5 % CO_2 challenge [M = 9.35, $t_{(10)}$ = 2.08, p = 0.6]; the FA group reported a significant increase in anxiety [M = 18.16, $t_{(10)}$ = 4.01, p = 0.002, dz = 1.21], and the control group reported the biggest increase in anxiety [M = 31.20, $t_{(9)}$ = 4.81, p = 0.001, ds = 1.52]. The control group reported significantly higher levels of anxiety vs. the OM group [$t_{(19)}$ = 2.28, p = 0.034, ds = 0.99]; there was a trend in the similar direction when comparing post CO_2 reported anxiety levels in the control vs. FA groups [$t_{(19)}$ = 1.74, p = 0.098, ds = 0.76]. There was a significant Group x Time effect on the secondary measure of state anxiety (STAI-S) [$F_{(2,29)}$ = 5.13, p = 0.012, $\eta2P$ = 0.26].
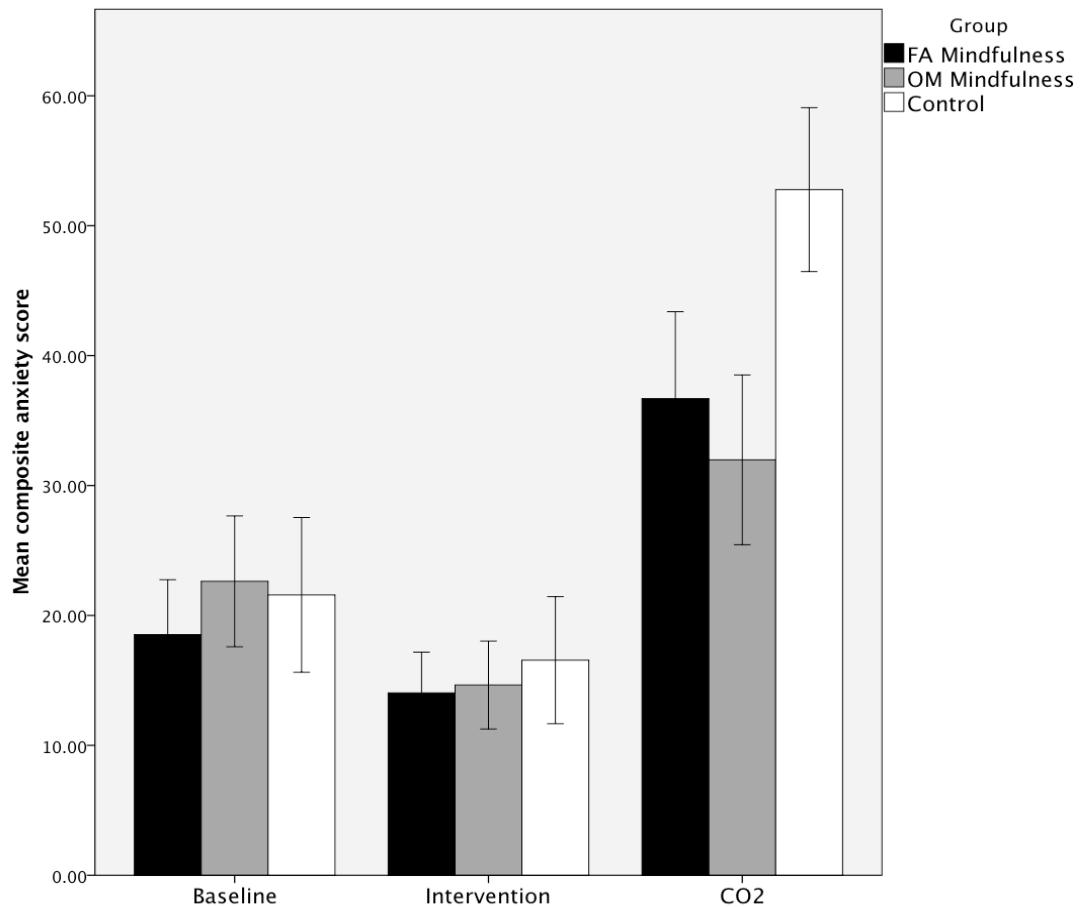
Figure 5.3   Effects of FA, OM and control interventions on mean composite anxiety scores

Supplementary follow-up 3 (Group) x 2(Time) ANOVA examined group differences in anxiety between i) baseline and post-intervention, and ii) post-intervention and post-Co2. These analyses supported findings reported in the main text. For example, groups did not differ in their anxiety at baseline (F<1) nor post-intervention (F<1) (nor change from baseline and post-intervention). All groups reported increased anxiety following Co2 vs. post-intervention (p's < .05), however groups significantly differed in anxiety post-CO2 characterised by FA and OM groups reporting less anxiety vs. RC (p's < .05).

5.3.4. Effects of focused attention (FA) and open monitoring (OM) on heart rate and blood pressure

Measures of arterial blood pressure (2 x Diastolic + Systolic)/3) and of heart-rate (Table 5.3 and Figure 5.4) were entered into separate mixed design ANOVA with Group (FA, OM, RC) as a between-subjects factor, and Time (baseline, post-intervention, post- CO_2 challenge) as a within-subjects factor (Table 5.3). The 7.5% CO_2 challenge was associated with increased heart rate [$F_{(2,29)}$ = 24.14, p < .001, $\eta2P$ = 0.62]; there was no significant between-groups difference in the magnitude of this increase [$F_{(4,58)}$ = 1.17, p = 0.33]. The 7.5% CO_2 challenge was associated with increases in blood pressure in each group [$F_{(2,29)}$ = 16.55, p < 0.001, η^2_P = 0.53]. Although there was a significant between-group difference on this measure, with a Group x Time interaction [$F_{(4,58)}$ = 3.05, p = 0.024, η^2_P = .17], this may have been related to higher baseline blood pressure in the control group compared to the mindfulness groups ([$t_{(19)}$ = 1.97, p = 0.06]. There were no other between-group differences at any time (t's < 1.26, p's > 0.22).

Table 5.3: Mean autonomic scores across time (baseline vs. post-intervention vs. post-inhalation)

	FA			OM			Control		
	Base	Post-Int.	Post-CO_2	Base	Post-Int.	Post-CO_2	Base	Post-Int.	Post-CO_2
MAP	83.2 (6.7)	86.7 (12.2)	94.9 (9.5)	84.8 (9.4)	83.8 (9.1)	91.3 (12.5)	89.7 (8.2)	81.7 (10.3)	90.6 (10.8)
	MAP: Group x Time ANOVA: $F_{(4,58)} = 3.05$, $p = .024$, $\eta p2 = 0.17$								
HR	68.0 (9.5)	70.4 (10.3)	90.0 (18.0)	73.1 (16.1)	70.8 (17.9)	83.0 (28.4)	74.2 (11.7)	71.5 (11.4)	86.1 (10.8)
	HR: Group x Time ANOVA: $F_{(4,58)} = 1.17$, $p = 0.33$								

MAP = Mean Arterial Pressure, HR = Heart Rate.
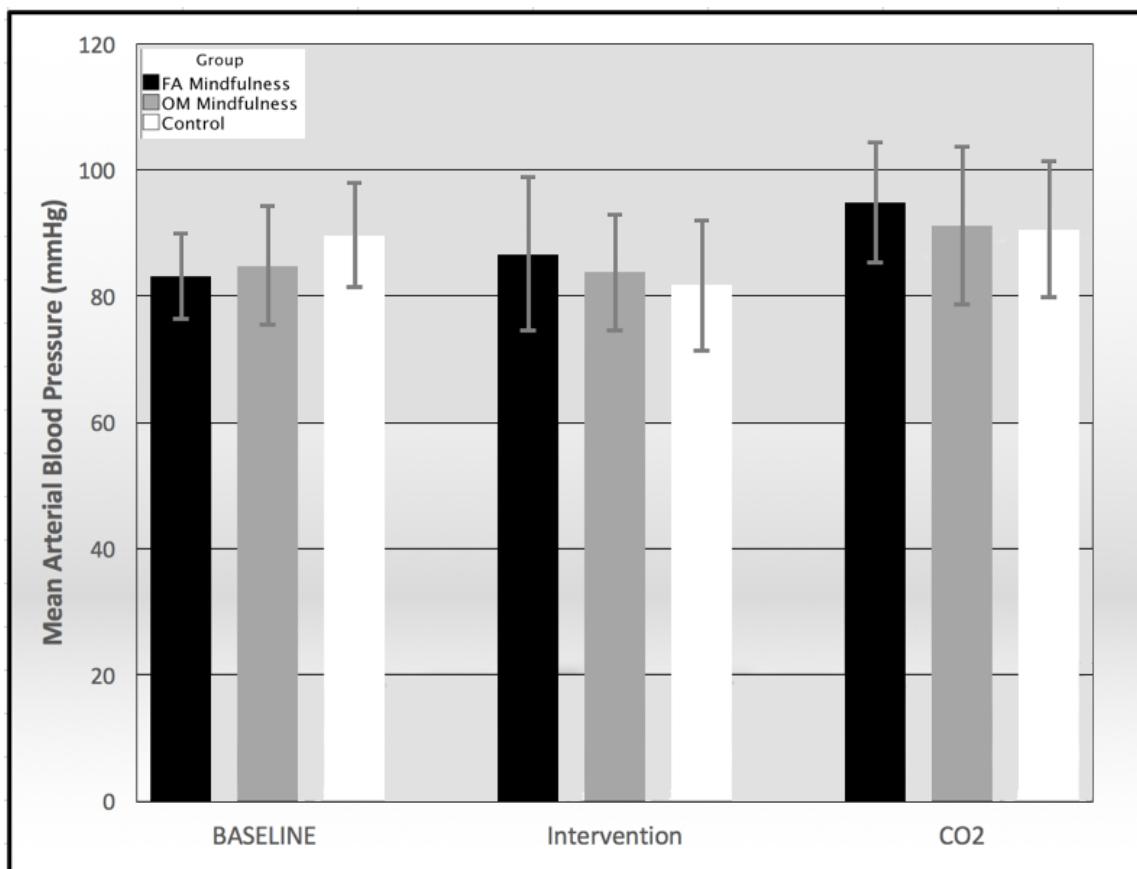
Figure 5.4   Effects of FA, OM and control interventions on mean arterial pressure (MAP) . MAP calculated as (2 x Diastolic + Systolic)/3).

5.3.5. Adverse outcomes and side effects

No adverse outcomes or side effects were reported during this study.

5.4. Discussion

This is the first study to demonstrate that components of contemporary mindfulness interventions can reduce anxiety in an experimental human healthy subject model of anxiety. Both FA and OM interventions were associated with significantly less increase in the anxiety levels reported after 20 minutes of 7.5% CO_2 inhalation; there was also a significantly reduced level of 7.5% CO_2-related anxiety in the OM group as compared against the control group, with a similar trend also observed in the FA group. This self-report anxiolytic effect was not associated with concurrent between-group differences in blood-pressure or heart rate. There was also no significant effect of mindfulness interventions on participants' performance on the anti-saccade task: neither saccade accuracy rate (reflecting attention control effectiveness), nor correct-saccade latency (a measure of task efficiency) showed any significant between-group differences. It seems that OM meditation has selective effects on 7.5% CO_2-related reported (subjective) anxiety, in the absence of effects on autonomic arousal or on the neuro-psychological impact of the 7.5% CO_2 challenge. FA seems to have similar, but less pronounced effects. OM meditation nurtures an attitude of acceptance and non-judgmental curiosity towards anything that arises within the field of awareness. OM encourages emotional regulation, which is strengthened by an open attitude towards emotional states and associated physical sensations[207]. In the context of the 7.5% CO_2 challenge,

OM practice may allow participants to actively engage in the task, while being less vulnerable to the anxiogenic effects of the challenge, including its physical manifestations such as increased heart-rate. In contrast, the FA practice as utilised in the study, encourages one-pointed concentration on the sensations of breathing. It may be that the observed anxiolytic superiority of OM over FA (and the lack of significant superiority of FA over relaxation control), are both related to technical aspects of the practice: participants often choose to observe the breath in the nose / mouth / throat areas – during the challenge, participants breathed through an oro-nasal mask, which may have interfered with their ability to concentrate in these areas, and may have actually increased the anxiogenic effect of the challenge by focussing awareness on the breath (which in this challenge can be seen as the source of anxiety). The anti-saccade task itself, may be problematic for FA practice, as it is inherently distracting participants away from the one-pointed attentional focus they are trying to maintain. Conceptually, OM practice, which encourages experiencing whatever comes up in the mind, may be more suited to facilitating habituation and anxiety extinction; whereas FA may be practiced as an avoidance of anxiogenic stimuli, and may therefore impede extinction. OM and FA reduced 7.5% CO_2-related anxiety increases without affecting autonomic measures of anxiety – in this their effects are similar to those of anxiolytic medication (e.g. benzodiazepines and SSRIs), which also reduce 7.5% CO_2-related reported anxiety, but not the associated autonomic responses[149,151]. Pharmacological interventions which reduce autonomic arousal (e.g. beta blockers such as propranolol) do not reduce 7.5% CO_2-related reported anxiety, even though they reduce the associated tachycardia[377]. It may be that effective treatments for anxiety (both psychological and pharmacological) uncouple the connection between the

subjective and autonomic responses to the 7.5% CO_2-challenge[127,143,378]; the mechanisms involved may be similar: the subjective anxiolytic effects of several classes of medication are partly mediated through prefrontal down-regulation of sub-cortical anxiety-related circuits (e.g. amygdala and locus-coeruleous). Increased prefrontal cortical activity and prefrontal-amygdala connectivity, with associated reduction of GAD symptoms is seen with mindfulness interventions[207]. This study did not demonstrate significant effects of a single session of mindfulness practice on attention control and selective attention (as measured using the anti-saccade task) in healthy, meditation-naïve participants. There is evidence that longer periods of practice are associated with larger effects on both cognitive control and autonomic arousal[333,369]. There is also evidence that in order to benefit from a short mindfulness intervention in terms of psychological and somatic anxiolytic response, individuals need to have high levels of baseline dispositional mindfulness[379]. The study employed a one-session mindfulness intervention, and was not powered to assess the effects of dispositional mindfulness on the subjective and/or somatic anxiolytic response to such a brief intervention. Our reported sample size of 10 + participants per group provides 75% power at an alpha level of 5% to detect a medium-large effect size (Hedge's g = 0.63, effects of MBSR on anxiety, Hofmann et al. 2010[373]). This study is possibly under-powered compared to earlier studies described in this thesis. The current study did not provide evidence that mindfulness improved performance on antisaccade performance accuracy, speed nor global measures of performance (e.g. overall antisaccade accuracy). We did not directly measure the effect of CO_2 on saccade performance vs. performance during air control condition (see Garner et al. 2011 for how this can be done[143]). Consequently future studies should examine the effect of mindfulness (vs.

control interventions) on 'CO$_2$-induced deficits' in antisaccade performance vs. performance during air inhalation.

5.5. Limitations

There were several limitations to this study – (these are further discussed in section 8.5.2):

The lack of an active control group – whereas the active intervention group received 10 minutes of guided mindfulness meditation instructions, the control group were simple instructed to relax.

The mindfulness training intervention was not associated with significant effects on participants' levels of dispositional mindfulness as measured using the MAAS and FFMQ.

5.6. Implications and future prospects

This study demonstrated that a single 10-minute intervention with either FA or OM meditation training is associated with significant anxiolytic effects in an experimental model of anxiety in healthy human subjects. OM was associated with more marked anxiolytic effects, and this may be an important finding in terms of developing therapeutic interventions that aim to improve acceptance and emotional openness towards anxiety symptoms, rather than focussing on distraction strategies. Future research aimed at exploring the effects of baseline dispositional mindfulness on the anxiolytic utility of brief mindfulness interventions may be helpful. It may also be prudent to explore alternative forms of FA practice that do not involve a breathing-focus, and to evaluate whether these may be better suited for the 7.5% CO$_2$ challenge.

The next two chapters describe an evaluation of tDCS as a potential treatment for anxiety – utilising parallel experimental designs to those used in chapter 4 and 5.

5.7. Funding

6. CHAPTER 6: TDCS AND ATTENTION NETWORKS

6.1. Introduction

6.1.1. Anxiety is associated with specific attentional characteristics

Anxiety is associated with distinct attentional patterns – these include reduced attentional control in the presence of negatively valenced and threatening stimuli[328], hypervigilance to threat and negatively valenced stimuli[329] and increased distractibility[328,330]. The attention control theory of anxiety describes a disruption of attentional resource-allocation balance, from 'top-down' (goal directed) towards 'bottom-up' (stimulus driven) attention[331]. These patterns may be associated with dysfunctional attention-control mechanisms underpinning pathological phenomena such as worry, anxiety and rumination. fMRI evidence lends support to the idea that anxiety is associated with reduced activity in frontal and/or prefrontal attention-regulating circuits[332].

6.1.2. Three attention networks

The complex phenomenon known as attention is made-up of several attentional components[90]. A number of sub-cortical and cortical networks interacting with each other, give rise to the group of processes underpinning attention[91]. In 1971 Posner and Boies put forward an early version of a three-network model[92]. This model maintains that there exist three demarcated attentional networks, each with its own structural and functional characteristics. Despite having its origins prior to the availability of extensive neuroimaging data, the model remains relevant albeit with some modifications. Modern imaging data lends further support to the model[94-96]. These

three networks can be viewed as aspects of an attentional organ system, incorporating histological, neuroanatomical, and functional components[97]. Different three-network models have been proposed over time[98,99], describing parallel, but not identical entities with different names[91]. The three attentional networks proposed by these models are currently termed: 'alerting', 'orienting', and 'executive'[100]. The **alerting attentional network** modulates alertness, which can be defined as the capacity to muster and sustain impending stimulus response-readiness[91,92], or as readiness for receiving information and responding to it[101]. Alertness can be divided into intrinsic (non-specific, endogenous, generalised level of arousal), and phasic (exogenous, task-related) components[102,103]. The alerting system has anatomical associations with right frontal and parietal areas[104], and with the CNS noradrenergic system[100,105,106]. There is an additional layer of complexity associated with the interaction between phasic (task-related) alertness, and intrinsic arousal: arousal is mediated by multiple systems[107] including right frontal networks (sub-cortical & cortical) coordinated by the anterior cingulate cortex[108]; these include frontal, thalamic, brainstem, inferior parietal, and anterior cingulate cortex structures[90]. Task-specific alerting may influence arousal levels via left hemisphere executive networks, and the right dorsolateral prefrontal cortex[91,100], as well as the thalamus, and the superior and ventro-lateral frontal gyrus[102]. The **orienting attentional network** is the most thoroughly understood attentional network[91]. Orienting (also referred to as selection or scanning) is defined as the capacity to separate particular items or strands of information out of the totality of available sensory inputs[91]. The orienting attentional system mobilises attention towards specific stimuli. Top-down (endogenous) orienting is driven by executive processes, while bottom-up (exogenous) orienting involves automatic capture of

attention by external stimuli[109]. Orientation can also be subdivided into overt and covert orientation (e.g. with or without eye movement) [110]. The concept of re-orienting refers to the alteration of attentional focus and direction in response to unexpected stimuli[111]. Areas associated with orienting functions include the superior parietal and temporal lobes, temporo-parietal junction, and frontal eye-fields[91]. There is evidence for 2 sub-networks: the first is top-down, dorsal network, directing attention towards goal-directed stimuli - it includes areas within the superior frontal and intra-parietal cortices. The second sub-network, is a bottom-up network, which includes areas within the inferior frontal and temporo-parietal cortices. This right, lateral ventral system can act as a stimulus-driven cut-off system, re-orienting attention towards salient, unexpected stimuli[69].   The **executive attentional network** is responsible for top-down, higher-level processes involving resolution of conflicts between competing computations or stimuli, and allocating attentional capacity to concurrently presenting stimuli and/or active regions. Executive network functions may include making decisions, detecting errors, cognitive and emotional regulation, suppression of habitual responses, and navigating danger or difficulty [87,91,112]. Anatomical regions associated with executive functions include the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex, locus coeruleus, and the ventral tegmental area[91,95,113].

6.1.3.   The Attention Network Test (ANT)

The Attention Network Test (ANT) is a computerised reaction time test first described by Fan and colleagues in 2002[100]. The ANT uses a combined cued reaction time[109] and flanker tasks[130] to independently measure the performance (Response Time and Error Rate) of the alerting, orienting and executive attention networks. The original version

of ANT repeatedly invites subjects to respond to a central arrow pointing either right or left; the arrow may be flanked by four additional distractor arrows – these may point in the same direction as the central arrow (congruent condition), or in the opposite direction (incongruent condition). Subjects are intermittently cued by temporal and/or spatial visual stimuli – providing alerting and orienting cues. Several modified versions of the ANT have been developed – allowing for instance to differentially assess re-orienting and orienting by contrasting reaction times and accuracy between validly cued stimuli, and stimuli presented following false (invalid) spatial cues[131]; another modified version allows the arrows to be presented to the right or left of the fixation point[132].

6.1.3.1. The Attention Network Test and anxiety

The relationship between anxiety and attention has long been recognised[133]. Attentional abnormalities associated with anxiety include hypervigilance[134], and attentional bias highlighting anxiety-related stimuli[135]. The spectrum of anxiety is often subdivided into state and trait anxiety. The effects of these subtypes of anxiety on attention have been proposed to be different[136] – in that state anxiety enhances the threat valence of a stimulus, whereas trait anxiety causes attention to be directed consistently towards potential sources of threat. State anxiety is triggered by situational factors, so is likely to be associated with bottom-up processes, whereas trait anxiety is related to personality factors and therefore likely to be more associated with top-down processes[73]. There is some evidence demonstrating that trait anxiety is associated with reduced executive control performance on the ANT: Pancheco-Unguetti and colleagues used a modified form of the ANT (ANT Interactions ANT-I) to

test subjects with high vs. low trait anxiety scores[73]. High trait anxiety subjects demonstrated significant deficiencies in executive control network performance. Stat anxiety showed associations with orienting network and alerting network over-functioning. Pancheco-Unguetti and colleagues[74] compared performance on the ANT-I between patients with anxiety disorders and healthy controls. Anxiety disorders were associated with executive attentional network dysfunction and with reduced efficiency in attentional disengagement from invalid cues – including emotionally neutral cues. Han and colleagues showed that adolescents with comorbid depression and anxiety disorder demonstrated a faster orienting response on the ANT when compared to depressed adolescents without comorbid anxiety disorder[138].

6.1.4. tDCS

Transcranial Direct Current Stimulation (tDCS) is a non-invasive brain stimulation modality, which changes cortical tissue 'excitability' as a result of applying a weak (0.5–2 mA) direct current via scalp electrodes overlying targeted cortical areas[253]. In contrast to other neuro-stimulation modalities, tDCS does not directly trigger action potentials in neuronal cells, but instead changes overall tissue excitability, and therefore may be more aptly regarded as a 'neuro-modulatory' rather than a neuro-stimulatory approach[232]. Cortical tissue underlying the anode (positive electrode) becomes hypo-polarised, and therefore hyper-excitable; areas underlying the cathode (negative electrode) become less excitable as the average resting potential becomes more polarised. The magnitude of these membrane polarisation changes is not in itself sufficient to directly cause neurons to fire[233]. These effects continue after electrical stimulation ceases, and a single application may be associated with tissue excitability

changes lasting up to a few hours[234,235,380]. These findings suggest tDCS is likely to be associated not only with transient membrane polarisation changes, but also with longer-lasting synaptic changes[296]. The mechanisms facilitating these tDCS after-effects may involve glutamatergic synapses and intra-cortical inter-neurons[90,381].

6.1.4.1.   tDCS and anxiety

In contrast to the growing evidence base for the use of transcranial direct current stimulation in depression, there is relatively little published research about its use in anxiety disorders[298]. To date, there are no published randomised controlled trials of tDCS in anxiety disorders. There is a limited number of published case studies describing the treatment of patients suffering from a range of anxiety disorders with tDCS (see Chapter 2).

6.1.4.2.   tDCS and Attention networks

There have been only a few studies of the effects of tDCS on attention. A study in patients with neurological neglect demonstrated that anodal tDCS at 1 mA over the affected PPC, and cathodal tDCS over the unaffected PPC, was associated with overcoming the ipsilateral line bisection bias[382]. Findings from studies in healthy controls of the effects of tDCS on attention include the following: 1. Anodal tDCS at 1mA over the posterior parietal cortex (PPC) changed the direction of attention towards the contralateral hemi space, cathodal stimulation had the opposite effect[382]. 2. Cathodal tDCS at 2.0 mA over the right intra-parietal sulcus (IPS) increased top-down control in a visual attention task[383]. 3.Anodal tDCS at 1.0mA over left dorsolateral prefrontal cortex (L-DLPFC) improved vigilance decrement in a simulated air traffic

controller task[384]. 4. Active vs. sham anodal tDCS at 2.0mA over right inferior frontal cortex (F10) was associated with improved object detection and ANT alerting network performance[247]. 5. Anodal tDCS over right PPC was associated with faster attentional orienting responses to contralateral (but not to ipsilateral) targets[385]. 6. Anodal (vs. cathodal) tDCS at 1.5mA over the pre-supplementary motor area was associated with increased inhibitory control in a stop signal task[386]. 7. tDCS at 1 mA over Dorsolateral Prefrontal Cortex is associated with improved performance on the Tower of London task[387].

6.1.4.3. tDCS and ANT

Two publications have described the effects of tDCS on the ANT. First, active vs. sham anodal tDCS at 2.0mA over right inferior frontal cortex (F10) was associated with improved object detection and ANT alerting network performance[247]. Second, Roy and colleagues recently found that tDCS at 1.5 mA over the right parietal cortex (but not the left parietal cortex or left dorsolateral prefrontal cortex) was associated with improving orienting network performance on the ANT. Right parietal cortex tDCS also selectively improved mean network efficiency for targets presented in the left (contralateral) visual field[90].

6.2. Aims

The evidence base for treating anxiety with tDCS is limited, as is our understanding of the effects of tDCS on attentional networks. There is evidence, linking dysfunction of attention networks with anxiety. This study aims to explore the effects of a single

session of active versus sham left DLPFC anodal tDCS with contralateral cathode placement, on attention network function in healthy volunteers. This could additionally clarify whether this intervention might be useful for targeting attention-related mechanisms underlying anxiety. The tDCS montage we selected consisted of the anode placed over L-DLPFC and the cathode over R-DLPFC. The rationale for selecting this montage was that anodal stimulation of the L-DLPFC is associated with mood improvement in depression[253], positive effects on cognition[388], enhanced emotional state processing in healthy subjects[389]. It was predicted that in comparison to sham tDCS, a session of active anodal L-DLPFC tDCS would significantly improve attention network function as measured using the ANT in healthy volunteers. The study by Roy and colleagues[90], which recently found that tDCS at 1.5 mA over the left dorsolateral prefrontal cortex was not associated with significant changes on the ANT had not been published at the time when we were formulating our hypotheses for this chapter.

6.3. Method

6.3.1. Participants

31 Participants were recruited and randomised into two groups - one participant was excluded from analysis due to being an extreme outlier on primary outcome measure accuracy – therefore only 30 participants were analysed: Active tDCS (n=15; five males and ten females; mean age = 20.8 years) or Sham tDCS (n=15; four males and eleven females; mean age = 21.5 years). See Figure 6.1 for consort diagram. Participants were volunteers aged 18-55 who were recruited via advertising. Exclusion criteria were: history of epilepsy, hypertension (above 140/90mmHg), or mental illness (including depression and/or anxiety), the presence of metal implants (including pacemakers,

dental implants etc.), current self-reported pregnancy or breastfeeding, weekly alcohol intake exceeding 50 units for males or 35 units for females. Candidates who had over the previous 8 weeks taken medication other than topical preparations, oral contraceptives, paracetamol, or aspirin, or whose body mass index (BMI) was outside the range of 18-28, were also excluded. Participants were initially screened using a telephone health screening tool. They were further screened on the day of the study session using the Mini International Neuropsychiatric Interview (MINI)[372] – this structured clinical interview is widely used to screen healthy volunteers for mental disorders using DSM IV[390] diagnostic criteria.

Figure 6.1: Consort diagram showing study design and progress of subjects through the trial.

6.3.2. Study design

This was a randomised, sham-controlled double blind between-subjects trial comparing the effects of a single session of active vs. sham tDCS on participants' mood and anxiety and on their performance on the ANT. The primary outcome measure was performance on the ANT. An *a priori* power calculation for a repeated measures three variable ANOVA (group X cue X congruence) was preformed. A sample size of n=20 (10 vs. 10) was necessary in order to detect an effect size (η^2_P = 0.1) using an alpha level of 0.05 and power of 0.8. The University of Southampton Research Ethics and Governance committee approved the test protocols, as well as the screening and recruitment processes.

6.3.3. Intervention with tDCS

The tDCS montage utilised was bi-frontal, with the anode placed over the L-DLPFC and the cathode over the R-DLPFC. This is the commonly used montage in modern tDCS trials in depression. Two tDCS stimulators were used; these were coded using numbered labels and pre-programmed to deliver either active or sham tDCS. Active tDCS was delivered using a 2mA current setting for 20 minutes. Sham tDCS was delivered using a brief (15 second) ramp-up, ramp-down protocol to simulate active stimulation and aid in blinding. Participants and staff members applying tDCS were blinded to active/sham tDCS allocation. The tDCS apparatus used in this study was the Magstim HDCkit (Handheld Direct Current). HDCkit consists of a programming unit, stimulator, and two electrodes (4 X 4cm). The electrodes are covered in a sponge envelope soaked in normal saline (0.9% NaCl).

6.3.4. Outcome measures

6.3.4.1. Primary outcome measure

6.3.4.1.1. Attention Network Test (ANT):

The Attention Network Test (ANT) is a computerised reaction time test first described by Fan and colleagues in 2002[100]. The ANT uses a combined cued reaction time[109] and flanker tasks[130] to independently measure the performance (Response Time and Error Rate) of the alerting, orienting and executive attention networks. The ANT was administered by using Inquisit 2 software, Millisecond Software, Seattle, WA. (www.millisecond.com). Participants were presented with a series of visual stimuli (See Figure 6.1) and tasked with responding as fast as they could by pressing buttons indicating whether the central arrow was pointing to the right or to the left. The arrows were preceded by "+" cues – these cues could be central, double, absent (no-cue) or spatial. The central (target) arrow was flanked by four distractor arrows, which could be pointing in the same direction (congruent distractors) or in the opposite direction (incongruent distractors). Congruence, target location, and direction were counter-balanced across the four cue types. Each participant was asked to undertake eight practice trials before going on to complete 128 experimental trials (32 trials per cue-type). Calculations of effects were conducted by subtracting Mean Response Times (RT) in msec. Data was cleaned by removing all data if RT > 1000msec. Alerting effect = Mean RT (Double cue trials) − Mean RT (No cue trials). Orienting effect = Mean RT (Spatial cue trials) − Mean RT (Centre cue trials). Executive Control effect = Mean RT (Incongruent trials) − Mean RT (congruent trials). Higher executive control scores suggest worse executive control performance, as the mean RT difference between the simpler congruous and more challenging incongruous trials increases.

6.3.4.2.   Secondary outcome measures:

6.3.4.2.1.   Spielberger State-Trait Anxiety Inventory[335]

The Spielberger State-Trait Anxiety Inventory is a widely accepted instrument for measuring both state and trait anxiety. It has been translated into more than thirty languages, and cited in over 3000 studies[313]. The Spielberger State-Trait Anxiety Inventory consists of 40 items, 20 aimed at assessing trait anxiety (Spielberger Trait Anxiety Inventory - STAI), and 20 measuring state anxiety (Spielberger State Anxiety Inventory - SSAI). Each item is a 4 point forced-choice Likert scale[314]. The state and trait scales each contain 2 factors: "anxiety absent" and "anxiety present". Trait anxiety is a tendency towards feeling anxiety, worry, discomfort, and stress; it describes a relatively stable characteristic or disposition, rather than a reaction to a particular set of conditions. The STAI addresses how subjects feel generally, commonly or usually. Items on the trait scale include statements such as: "I am content", "I have disturbing thoughts". Each item on the trait scale is scored: 1 almost never; 2 sometimes; 3 often; 4 almost always. State anxiety is a transient response to a set of conditions perceived as threatening or dangerous. It can include components such as autonomic arousal, and feelings of fear, discomfort, or nervousness. The SSAI measures how subjects feel "right now, at this moment"[314]. Items on the state scale include statements such as: "I am calm", "I am worried". Each item on the state scale is scored: 1 not at all; 2 somewhat; 3 moderately so; 4 very much so. Scores generated on each scale can range from 20 – 80, higher scores reflect more intense anxiety[314]. The Spielberger State-Trait Anxiety Inventory was found to have good internal consistency (average αs > 0.89)[314]. The test-retest reliability at different time points of the trait scale (STAI) is also good

(average r = 0.88)[315]. The test-retest reliability of the state scale (SSAI) at different time-points is of course lower (r = 0.70).

6.3.4.2.2. Visual Analogue Scale (VAS)

This can be used to measure a construct across a continuum of values, when direct measurement is difficult or impossible to achieve. Examples of characteristics that can be measured using a VAS include subjective pain, intensity of emotional experience, or attitudinal response. It is important for the construct to be perceived as a continuum rather than a set of discrete steps or values. The VAS is presented as a 100mm horizontal line, stretching between two anchor statements defining the end points of a continuum, with optional additional statements situated next to the line, indicating waypoints along the continuum. The subject is tasked with indicating their position along the continuum by marking the line. Measuring the distance in millimeters from the left end of the line to the marked point derives the score. In this study, we presented subjects with 6 VAS scales items relating to subjective feeling: 1 "alert"; 2 "worried"; 3 "happy"; 4 "relaxed"; 5 "anxious"; 6 "feel like leaving". Each was anchored by 2 statements: from left:" Not at all", to right: "All the time". The 6 scales make up 3 groups: cognition (1), positive affect (3, 4), and negative affect (2, 5, and 6). VAS scales are widely used for measuring pain and other psychological states, and have also been validated in anxiety[357]. The evidence suggests that VAS scales perform well in comparison to subjective measures such as the Likert and Borg scales[358,359].

6.3.4.2.3. GAD-7[391]

The GAD-7 is a 7 item questionnaire validated and extensively used for screening and

assessing the severity of Generalised Anxiety Disorder (GAD) in clinical practice and research[391]. The scale prompts subjects to respond to the question "Over the last 2 weeks, how often have you been bothered by the following problems?: 1 Feeling nervous, anxious or on edge; 2. Not being able to stop or control worrying; 3. Worrying too much about different things; 4. Trouble relaxing; 5. Being so restless that it is hard to sit still; 6. Becoming easily annoyed or irritable; 7. Feeling afraid as if something awful might happen". Each item is scored 0-3 on a Likert-like scale offering the following response choices: 0:"Not at all"; 1: "Several days"; 2: "More than half the days"; 3: "Nearly every day". Anxiety severity cutoffs used are : Mild anxiety ≥5, Moderate anxiety ≥ 10, Severe anxiety ≥ 15. A GAD-7 score of ≥ 10 has sensitivity of 89% and specificity of 82% for GAD[392]. In this study, a modified the GAD-7 was used to assess the severity and change of anxiety comparing pre and post tDCS stimulation scores. Participants were asked to rate their experience over "the last 20 minutes" rather than over the last 2 weeks as in the original GAD-7. A Visual Analogue Scale (VAS) was used, anchored from left "Not at all" to right "All the time".

6.3.4.2.4.   The Penn State Worry Questionnaire (PSWQ)[337]

The PSWQ is a well-established self-report instrument used to measure subjects' worry trait. The PSWQ contains 16 items relating to worry; each item is scored on a 5 point Likert scale ranging from 1 "Not at all typical of me" to 5 "Very typical of me". Eleven items are positively scored – for example: "I am always worrying about something" , and 5 items are scored in reverse – for example: "I do not tend to worry about things". Higher scores are correlated with increased tendency to worry. PSWQ demonstrates good internal consistency (α = 0.90)[340], and test-retest reliability[341].

6.3.4.2.5. Anxiety Sensitivity Index (ASI)[393]

Anxiety sensitivity is a concept describing the fear of physiological, psychological, or behavioural manifestations of anxiety ("fear of fear"), based on beliefs that these sensations predict adverse physical, psychological, or social consequences[394]. Anxiety sensitivity is seen as possessing a cognitive dimension rooted in dysfunctional beliefs about the meaning of anxiety manifestations, and therefore goes beyond simple Pavlovian conditioning arising out of aversive anxiety experiences. Anxiety sensitivity is seen as preceding (and possibly even predicting) the onset of anxiety disorders[395]. The ASI is one of the most widely used instruments measuring anxiety sensitivity in clinical and non clinical populations[396]. The ASI is an 18 item questionnaire comprised of three 6 item subscales relating to physical, cognitive and social concerns. The ASI demonstrated good internal consistency and test-retest reliability (p=0.72; r = 0.75)[393,396,397]. In this study the ASI was utilised to assess potential interaction between subjects' somatic anxiety sensitivity and reported tDCS adverse effects.

6.3.4.2.6. Positive and Negative Affect Scale[374]

The PANAS is a 20 item questionnaire assessing affect along a 5-point Likert scale ranging from 1 "Very slightly or not at all" to 5 "Extremely". The PANAS is composed of 2 10-item sub-scales: Positive (PANAS-P) and Negative (PANAS-N). Both sub-scales demonstrate good internal consistency: PANAS-P α = 0.89, PANAS-N α = 0.85. Each item introduces an emotion (e.g. excited, enthusiastic, ashamed, hostile) and invites the subject to rate the extent to which they feel this emotion. Higher scores on the PANAS indicate more positive affect. This study used a PANAS state version, asking

subjects to rate their emotions "in the past 20 minutes".

6.3.4.2.7.   Attentional Control Scale (ACS)[114]

The ACS is a 20-item self-report measure assessing the ability to maintain attentional control in the presence of distractors such as environmental stimuli, concurrent tasks, and emotional states. The ACS presents items such as "When I am reading or studying, I am easily distracted if there are people talking in the same room", and "When trying to focus my attention on something, I have difficulty blocking out distracting thoughts"; it then asks subjects to rate themselves along a 4 point Likert frequency of experience scale ranging from 1 "Almost never" to 4 "Always". The ACS demonstrates good internal consistency using a two factors Focusing (α= 0.82) and Shifting (α = 0.71) sub-scales[342].

Figure 6.1 The Attention Network Test (adapted from Garner et al, 2011)[127]

6.3.5. Study workflow

Recruitment of participants was via advertisement within the university. Potential

participants underwent a telephone screening, guided by inclusion and exclusion

criteria. Those who were deemed suitable were invited to a test session and

instructed to avoid alcohol and moderate their caffeine intake during the 24 hours

leading up to the session. The test session itself lasted about 2 hours and was

conducted at the research laboratory at the Academic Unit of Psychology, Highfield

Campus, and University of Southampton. On arrival to the test session, participants

provided informed consent for taking part in the study. They went on to undergo an

additional screening session which included cardiovascular screening (systolic and

diastolic blood pressure, and heart rate) as well as screening for DSM IV disorders using the Mini International Neuropsychiatric Interview (MINI)[372]. Participants who were eligible following this additional screening, went on to undergo an additional set of baseline heart rate and blood pressure measurements, as well as completing a series of assessment questionnaires: STAI, PANAS, GAD-7, ASI, and VAS. Participants then went on to undergo a session of tDCS stimulation, they were blinded as to whether this was an active or sham tDCS session. Participants were instructed to relax in a seated position, and to avoid movement for the duration of tDCS stimulation. tDCS was delivered for 20 minutes: active tDCS using 2mA current setting, or sham tDCS using a 15 second 2mA ramp up. The anode was placed over the left DLPFC; the cathode was placed over the right DLPFC. The electrodes were taken off at the end of the stimulation period. Participants underwent a further measurement of heart rate and blood pressure, as well as completing a set of peak effect questionnaires: GAD-7, VAS, STAI (State), and PANAS. At that point participants went on to undergo a 20 minute Attention Network Test (ANT). Following completion of the ANT, participants were instructed to complete the ACS and PSWQ. Participants were then debriefed before departing. Participants were followed up 24 hours after the test session, invited to comment and express any queries, and asked about adverse effects. None of the participants reported any adverse events.

6.4. Results

6.4.1. Participants

Thirty participants completed test sessions. One participant was excluded from all analyses due to being an extreme outlier in respect to task accuracy on the primary outcome measure (ANT accuracy = 0.70). Data from 30 participants was therefore analysed. Independent sample t-tests demonstrate that there were no statistically significant differences in baseline characteristics between the two groups (active vs. sham tDCS). Baseline group characteristics are outlined in Table 6.1.

Table 6.1: Comparison of participant demographics and characteristics at study entry for active and sham tDCS groups

	Active tDCS		Sham tDCS			
	n=15		n=15			
	M	SD	M	SD	t(28)	*p*
Gender (F:M)	10:5		11:4			
Age (years)	20.8	1.8	21.5	2.9	0.69	*p=0.50*
BMI (kg/m^2)	22.01	2.86	22.62	2.27	0.62	*p=0.54*
STAI-T	36.13	8.46	33.73	7.00	0.85	*p=0.40*
GAD-7	22.07	16.55	24.19	12.84	0.39	*p=0.70*
ASI	32.67	9.15	32.73	9.73	-0.02	*p=0.99*
ACS	51.53	6.69	50.20	9.51	0.44	*p=0.66*
PSWQ	47.00	10.54	45.00	26.11	0.28	*p=0.79*

BMI = Body Mass Index; STAI-T = Spielberger State-Trait Anxiety Inventory – Trait sub-scale; ASI = Anxiety Sensitivity Index; ACS = Attentional Control Scale; PSWQ = Penn State Worry Questionnaire

6.4.2. Attention Network Test (ANT) and transcranial direct current stimulation(tDCS)

6.4.2.1. Comparison of global Error Rate and mean Reaction Times between the active and sham tDCS groups on the ANT task.

Table 6.2 describes a comparison between global error rates (error rate = 1 – accuracy) and mean reaction times for the active and sham tDCS groups. There were no statistically significant differences between the active and sham tDCS groups in the global error rates and mean reaction times on the ANT task. One session of active

anodal left DLPFC tDCS did not therefore significantly influence global accuracy or global reaction times on the ANT task.

Table 6.2: Comparison of global error rates (ERs) and reaction times (RTs) for active and sham tDCS groups on the ANT task

	Active tDCS		Sham tDCS			
	n=15		n=15			
	M	SD	M	SD	t(28)	p
Global Error Rates	0.04	0.04	0.02	0.02	-1.56	p=0.13
Global Reaction Times (msec)	462.10	62.57	501.94	48.56	-0.88	p=0.43

6.4.3. Mean Reaction Times (RTs) across the four ANT task cue types X cue congruence condition for active and sham tDCS groups

There were no statistically significant differences in mean reaction times (RTs) across the four ANT task cue types X cue congruence condition between the active and sham tDCS groups (Table 6.3).

An omnibus mixed model Analysis of Variance (ANOVA) [group (2) X cue type (4) X congruence (2)] demonstrated the following significant Response Times (RTs) effects:

- A statistically significant main effect of cue type (F(3,28) = 22.85, p <0.001): The mean RTs in Spatial cue trials (497.97 msec) were shorter than mean RTs in the central cue (521.57 msec; p<0.01) and double cue (523.82 msec; p<0.01) trials.

- A statistically significant main effect of congruence (F(1,28) = 336.94, *p* <0.001): Congruent trial mean RTs (480.37 msec) were shorter than incongruent trial mean RTs (562.62 msec).

- A statistically significant interaction between tDCS group and congruence F(1,28) = 4.27, *p* <.05): Active tDCS was associated with shorter mean RTs on congruent trials (475.37 msec) than on incongruent trials (548.36 msec) in comparison to sham tDCS (congruent trial mean RT = 485.37 msec, incongruent trial mean RT = 576.88 msec).

Table 6.3: Comparison of mean reaction times (RTs) (msec) across four ANT task cue types X cue congruence condition for active and sham tDCS groups

	Active tDCS		Sham tDCS			
	n=15		n=15			
	M	SD	M	SD	t(28)	*p*
No cue *congruent*	501.5	89.6	513.3	79.4	- 0.38	*p=0.71*
No cue *incongruent*	558.2	91.9	597.4	75.5	- 1.28	*p=0.21*
Central cue *congruent*	472.5	101	482.6	75.4	- 0.31	*p=0.76*
Central cue *incongruent*	557.6	84.0	573.6	62.3	- 0.59	*p=0.59*
Spatial cue *congruent*	466.1	82.8	473.7	75.0	- 0.26	*p=0.79*
Spatial cue *incongruent*	518.6	76.0	533.4	63.4	- 0.58	*p=0.57*
Double cue *congruent*	461.3	87.6	471.8	67	- 0.37	*p=0.715*
Double cue *incongruent*	559.0	92.0	603.1	60.0	- 1.56	*p=0.13*

6.4.4. Comparison of Alerting, Orienting and Executive Control attention network scores on the ANT between active and sham tDCS groups.

Alerting, Orienting and Executive Control attention network function as assessed on the ANT was compared across the active vs. sham tDCS groups. Independent sample t-tests yielded the following results (see Figure 6.2):

- Statistically significant Executive Control network performance superiority following active vs. sham tDCS ($t(1,28) = 2.07$, $p<0.05$).

- Non-significant difference in Alerting network performance between active vs. sham tDCS ($t(1,28) = 0.18$, $p=0.86$).

- Non-significant difference in Orienting network performance between active vs. sham tDCS ($t(1,28)= -0.19$, $p=0.85$)
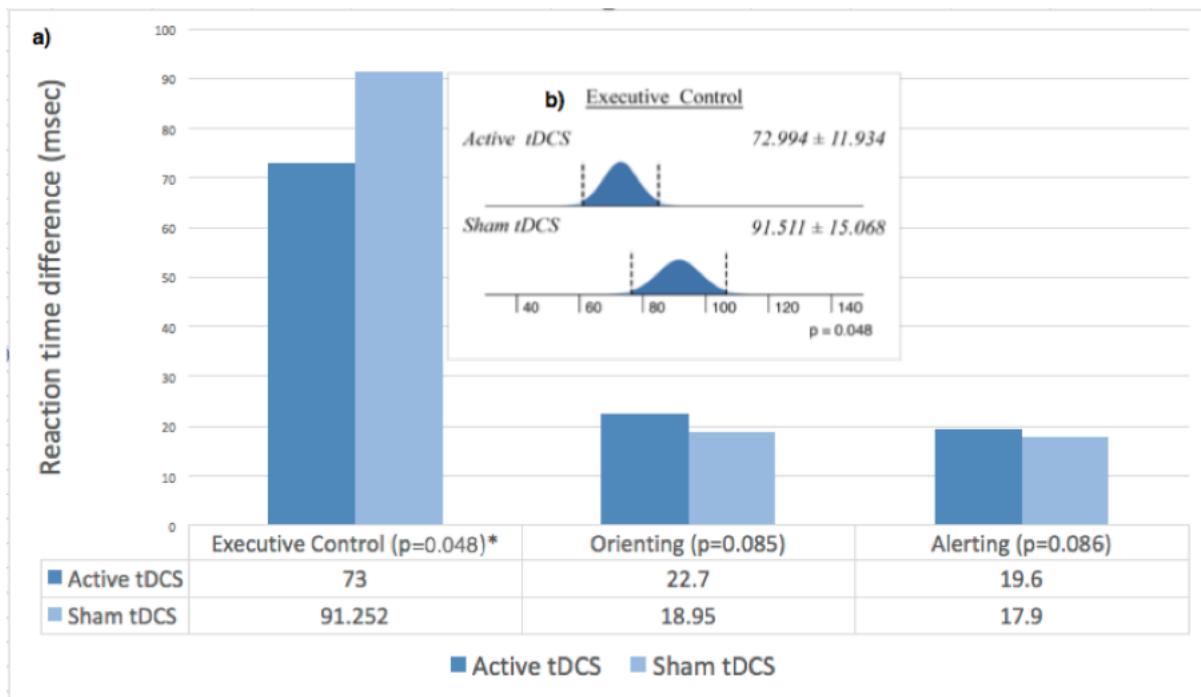
Figure 6.2 (a) Differences in attention network function following active vs. sham tDCS (b) t-test comparing ANT executive control after active vs. sham tDCS.

Additional ANOVAs were performed to test whether target congruence moderated the differences in Orienting and Alerting network function between the active vs. sham tDCS groups. Group (2) X Congruence (2) ANOVA on Alerting yielded a significant effect of Congruence on Alerting ($F_{(1,28)}=17.28$, $p <0.001$). Group (2) X Congruence (2) ANOVA on Orienting yielded a significant effect of Congruence on Orienting ($F_{(1,28)}=11.71$, $p<0.01$). Congruent trials were associated with significantly better Alerting and Orienting performance than incongruent trials. tDCS did not affect Alerting or Orienting network performance – there were no significant effects of tDCS group. There were no significant tDCS group X congruence interactions (F's <0.01, *p*'s >0.95).

6.4.5. Self-reported affect and anxiety

As shown in Table 6.4, there were no statistically significant differences in self-reported affect and anxiety before and after active vs. sham tDCS stimulation. A 2x2 mixed design ANOVA using a between-subject factor of tDCS group and a within-subject factor of time (pre/post stimulation) yielded significant main effects of time for VAS negative affect ($F(1,28) = 4.61$, $p=0.04$, $\eta^2_P=0.14$), PANAS positive affect ($F(1,28) = 6.67$, $p=0.02$, $\eta^2_P=0.19$), and VAS cognition ($F(1,28) = 26.24$, $p<0.001$, $\eta^2_P=0.48$). There were no additional significant main effects or interactions ($F's(1,28) < 3.60$, $p's>0.07$). There were no significant main effects of group and no time x group interaction for VAS positive affect, PANAS negative affect, STAI-State, or GAD-7 ($F's (1,28) < 3.41$, $p's>0.08$). A single session of active tDCS vs. sham tDCS was not associated with statistically significant differences in self-reported affect or anxiety. Participants in both active and sham tDCS groups reported reductions in both positive affect (assessed by PANAS) and negative affect (assessed by VAS), as well as cognitive blunting immediately after a single session of active or sham DLPFC tDCS.

Table 6.4: Self-reported affect and anxiety before and after active vs. sham tDCS stimulation

		Active tDCS		Sham tDCS			
		n=15		n=15			
		M	SD	M	SD	t(28)	*p*
PANAS positive	Pre-stimulation	23.73	6.23	27.80	6.21	-1.79	*p=0.08*
	Post-Stimulation	21.40	6.09	24.60	5.47	-1.51	*p=0.14*
VAS positive	Pre-stimulation	100.90	22.54	114.97	22.41	-1.71	*p=0.10*
	Post-Stimulation	97.93	28.67	109.10	18.90	-1.26	*p=0.22*
PANAS negative	Pre-stimulation	12.13	2.30	12.13	2.56	<0.01	*p=0.99*
	Post-Stimulation	11.33	2.00	12.80	5.93	-0.91	*p=0.37*
VAS negative	Pre-stimulation	32.31	25.68	32.82	21.30	-0.06	*p=0.95*
	Post-Stimulation	21.70	15.46	27.36	20.85	-0.84	*p=0.41*
STAI-State	Pre-stimulation	34.80	10.41	29.73	10.26	1.34	*p=0.19*
	Post-Stimulation	33.20	8.09	29.20	5.72	1.56	*p=0.13*
GAD-7	Pre-stimulation	15.95	15.06	18.02	11.77	-0.42	*p=0.68*
	Post-Stimulation	14.53	14.78	14.38	13.04	0.03	*p=0.98*
VAS cognition	Pre-stimulation	94.73	20.08	106.60	27.42	-1.35	*p=0.19*
	Post-Stimulation	68.73	25.40	84.47	35.16	-1.41	*p=0.17*

6.4.6. Blood pressure and heart rate (table 6.5)

2x2 ANOVA demonstrated a significant main effect of time ($F(1,28)$ = 16.26, *p* <0.001, η^2_P=0.37), but no significant main effect of group, nor a significant interaction time x group (F's$(1,28)$ < 2.34, *p*'s>0.14). Heart rate post stimulation was significantly lower than pre-stimulation across both active and sham tDCS groups. Similar analyses of systolic and diastolic blood pressure failed to demonstrate significant main effects or

interactions (F's(1,28) < 0.95, *p*'s>0.34).

Table 6.5: Blood pressure and Heart rate before and after active vs. sham tDCS stimulation

		Active tDCS		Sham tDCS			
		n=15		n=15			
		M	SD	M	SD	t(28)	*p*
Systolic blood pressure (mmHg)	Pre-stimulation	118.87	13.86	115.60	12.27	0.66	*p=0.52*
	Post-Stimulation	117.73	22.98	113.67	11.34	0.62	*p=0.54*
Diastolic blood pressure (mmHg)	Pre-stimulation	67.33	9.85	68.93	6.93	0.02	*p=0.99*
	Post-Stimulation	71.93	23.38	66.47	8.55	0.85	*p=0.40*
Heart Rate (BPM)	Baseline	76.67	9.45	73.27	10.67	0.92	*p=0.36*
	Post-tDCS	68.67	11.56	69.67	9.44	-0.26	*p=0.80*

6.4.7. Adverse outcomes and side effects

No adverse effects were reported by participants in this study either during tests sessions or at 24 hour follow up.

6.4.8. Integrity of masking

Participants were asked to guess their group allocation at the end of the test session by crossing a box in answer to the question: "Do you think you received the active tDCS or the placebo/inactive tDCS?" 80% (12/15) of participants in the active tDCS group and 80% (12/15) of participants in the sham tDCS group guessed their allocation correctly.

6.5. Discussion

This is the first study demonstrating significant effects of anodal left dorsolateral prefrontal cortex tDCS on attentional network performance using the ANT. Roy et al. [398] did not find significant effects of anodal left dorsolateral prefrontal cortex tDCS on attentional network performance using the ANT. Impaired executive control performance on the ANT was previously found in high-anxiety cohorts[74]. This study demonstrated significant enhancement of executive control attentional network function following a single session of active tDCS using 2mA for 20 minutes, when compared to sham tDCS in healthy human volunteers. Previous studies in healthy subjects involving tDCS stimulation of DLPFC suggest that targeting the DLPFC may be of utility in modifying attention. There are reports of enhanced emotional face identification[389] and improved selective attention on a Sternberg task, with a suggestion that tDCS effects on working memory might be mediated by a specific effect on selective attention[399]. There is also evidence to suggest that tDCS of the DLPFC can affect attention bias modification[400].

6.5.1. Attentional network effects

The executive control network is involved in allocating attentional/cognitive resources to competing attentional demands; in particular, in situations involving conflicts between concurrent tasks or between task-relevant and task-distracting stimuli. Participants in both groups responded more slowly to incongruent than to congruent trials on the ANT – this reflects the added cognitive complexity required for generating correct responses following incongruent cues; however, when compared to participants receiving sham tDCS, participants who were exposed to active tDCS

demonstrated less slowing associated with incongruent cues. This implies that their executive control networks were functioning more efficiently. The improvement in executive control function was not accompanied by significant changes in the alerting nor the orienting network function. When compared to participants who received sham tDCS, participants who received active tDCS did not respond more quickly to non-cued vs. double-cued trials (alerting network), nor did they respond more quickly to spatial vs. central cues (orienting network). These results suggest that tDCS applied to the left DLPFC had a selective, as opposed to a global effect on attentional network function. This pattern of selective effects of DLPFC tDCS on executive but not on orienting or alerting networks is of particular interest as trait and state anxiety have been shown to affect ANT patterns differently: state anxiety is associated with executive control impairment, whereas trait anxiety is associated with deficits in orienting network function[73]. The selective effects of DLPFC tDCS on attentional networks may be related to the evidence that the three attentional networks are each associated with particular brain region activation: The executive control network is associated with Lateral Prefrontal Cortex and ACC[95,401], and therefore is likely to be affected by tDCS aimed at the DLPFC. The orienting network is associated with frontal eye fields and parietal lobe activation[95,402]; whereas the alerting network is associated with activation in frontal and parietal areas[95] – these networks are therefore less likely to be modulated by tDCS stimulation aimed at the DLPFC. Consistent with this argument is a study describing tDCS stimulation of the right inferior frontal cortex[247], which reported enhanced alerting network performance on the NT in healthy subjects tasked with a task assessing learning within a complex environment.

6.5.2. Effects on affect and anxiety

This study demonstrated no significant effects of active vs. sham tDCS on self-reported measures of anxiety or affect. This suggests that the observed effects on executive control were not mediated by changes in affect or anxiety. However, the effects observed on attention may be associated with downstream improvement in affect and anxiety when tDCS is delivered as a clinical intervention, involving repeated stimulation as a part of a course of treatment. This study was not designed to demonstrate this effect as it involved only a single stimulation and assessment session. The experimental design did not allow for an opportunity to offer repeated stimulation, which could potentially translate a change in executive function into a longer lasting effect on more stable constructs like mood and trait anxiety, as opposed to their more transient counterparts: affect and state anxiety. The population recruited to this study actively excluded participants with a history of anxiety or mood disorders. It is therefore not possible to comment on the potential mechanistic connection between the demonstrated effects of a single session of tDCS on executive network function, and the well-established effects of a course of tDCS in major depression. The finding of lack of significant effects of active vs. sham tDCS on affect or anxiety in healthy subjects following a single session of tDCS is in line with previous findings in the literature[389,403]. The study demonstrated mixed effects on affect in both active and sham tDCS groups: Both the PANAS positive subscale score and the VAS negative affect score decreased post stimulation. This may be related to a baseline trend towards increased PANAS positive affect in the sham vs. active tDCS group. Despite the difficulty of blinding in this study, participants randomised to the active tDCS arm did not demonstrate demand characteristics in terms of reported affect or state anxiety.

6.5.3. Autonomic arousal

This study did not demonstrate significant effects of active vs. sham tDCS on measures of autonomic arousal – heart rate or blood pressure – the observed improvement in executive control following active tDCS was therefore not mediated by changes in autonomic arousal. The absence of acute effects on blood pressure or heart rate after tDCS stimulation is in line with previous reports in the literature[404-406]. Participants in both active and sham tDCS groups demonstrated a significant reduction in heart rate between the pre stimulation to post stimulation measurements. It is likely that this effect is attributable to acclimatising to the laboratory environment, as well as to potential relief about the lack of adverse experiences during the tDCS stimulation session.

6.6. Implications and future prospects

This study implies that a single session of anodal tDCS stimulation over the left DLPFC is associated with significant enhancement in executive control performance in normal subjects. These findings have a range of implications:

- The therapeutic effects of tDCS in depression suggest a dose-response curve in terms of intra-session factors (e.g. current setting, session duration) and factors external to the session (including inter-session interval, number of sessions, tDCS montage etc.). Modifications of some of these factors may enhance the magnitude as well as the stability over time of the enhancement in executive function.

- Potential therapeutic effects in anxiety disorders: Anxiety disorders are associated with attentional network dysfunction – in particular, trait anxiety is

associated with orienting network deficits, while state anxiety is associated with executive control impairments [74]. It is therefore possible that combining tDCS interventions that target both executive and orienting network performance, may deliver more effective treatment for anxiety disorders by targeting both trait and state anxiety.

- Potential therapeutic effects in other disorders associated with executive dysfunction. A range of disorders are associated with impaired executive functions[407] including attention deficit and hyperactivity disorder (ADHD)[408], frontal and Alzheimer's dementia[409,410], some learning disabilities[411], and schizophrenia[412]. tDCS of DLPFC may have utility in these disorders in terms of improving executive function.

- Potential for improving executive functions in healthy individuals: modern society presents an increasingly competitive landscape in academic and occupational settings. There are potential advantages for healthy individuals improving their cognitive performance by enhancing their executive function. There is evidence to show that students and workers are already using prescription medication to enhance executive function and memory – these include amphetamines, modafinil and acetylcholinesterase inhibitors[413]. There is also a growing recognition that tDCS may represent another potential modality for enhancing cognitive performance in healthy individuals[414-416], it is also important to consider that cognitive enhancement may also have adverse effects – including cognitive adverse effects[417].

There is a clear need for developing the evidence base for tDCS in anxiety disorders. As described in the literature review in chapter 1, clinical trials of tDCS in people with anxiety disorders are rarely reported in the literature. This study develops our understanding of the effects of tDCS stimulation in healthy individuals on attention networks implicated in anxiety.

The next chapter describes a study offering a further step towards developing a tDCS intervention that may be helpful for treating people with anxiety disorders. This study tests tDCS stimulation in healthy individuals subjected to conditions simulating anxiety states utilising the 7.5% CO_2 inhalation model of generalised anxiety disorder (GAD), and the anti- saccade task as a measure of cognitive effectiveness and efficiency in exercising top-down control in order to override a reflexive tendency to look towards a stimulus appearing abruptly in peripheral vision.

6.7. Limitations

There were several limitations to this study – (these are further discussed in section 8.5.3):

The tDCS montage used in this study is likely to be preferentially selective towards modulating the executive control network, rather than the orienting / alerting networks.

The tDCS studies utilise a single session intervention, which may not be sufficient to change anxiety levels – particularly in a cohort of well volunteers.

6.8. Funding

7. CHAPTER 7: THE EFFECTS OF A SINGLE SESSION OF TDCS ON ATTENTION CONTROL IN THE 7.5% CO$_2$ CHALLENGE - A NOVEL EXPERIMENTAL HUMAN MODEL OF ANXIETY

7.1. Introduction

Chapter 6 described a study comparing the effects of a single session of active vs. sham DLPFC tDCS on attention network function, as measured using the ANT, in healthy volunteers. The study found that a single 20 minute session of active tDCS was associated with a enhanced executive control (but not alerting or orienting) network performance, compared to sham tDCS. The study did not find significant differences in autonomic arousal, affect, or anxiety, between the active and sham tDCS groups.

Chapter 5 described a study comparing the effects of a single session of guided mindfulness practice (FA vs. OM) vs. relaxation control, on attention control, measured using the modified emotional anti-saccade task, in the 7.5% CO$_2$ challenge. The study found that OM, and to a lesser extent, FA practice was associated with reduction in reported subjective anxiety during the 7.5% CO$_2$ challenge, in the absence of autonomic arousal differences. There were no significant between-group differences in attention control on the anti-saccade task.

The current study employs the same tDCS intervention used in Chapter 6, and the same experimental design as in chapter 5, using the 7.5% CO$_2$ challenge to model anxiety, while measuring attention control using the modified (emotional) anti-saccade task.

7.1.1. The 7.5% CO_2 challenge

As described in detail in chapters 1 and 5, inhalation of air containing elevated concentrations of carbon dioxide (CO_2) has long been known to induce symptoms of anxiety and panic[144], and has been used to this effect in healthy subjects[145,146]. Inhalation of concentrations of CO_2 greater than 7.5% is associated with a range of subjective and objectives manifestations of anxiety. Physiologically, there are signs of autonomic arousal including elevated blood pressure, pulse rate and sweating. Healthy subjects describe feelings of anxiety, tension and fear[149-151]. The effects of 7.5% CO_2 challenge are less pronounced than the panic symptoms associated with inhaling 35% CO_2[365].

Bailey and colleagues examined the 7.5% CO_2 model as a potential experimental model of GAD in two separate studies of healthy volunteers: in study 1 subjects were given a single dose of lorazepam, and in study 2 participants were treated with 21 days of the SSRI paroxetine. The authors concluded that the 7.5% CO_2 model is sensitive to a treatment with proven effect in GAD, and that this supported the model's utility as an experimental model of GAD in healthy volunteers[149]. This conclusion is further supported by evidence from GAD patients in whom the clinical picture of GAD is reproduced when exposed to a 7.5% CO_2 challenge[152]. The 7.5% CO_2 model of anxiety in healthy subjects has been shown to be particularly sensitive to the effects of benzodiazepines and a corticotrophin releasing factor (CRF1) antagonist [157], less sensitive to the effects of SSRIs, and not sensitive to venlafaxine or pregabalin[139] – this may be related to dosing and timing issues in the studies, but may also relate to

limitations of the model. To date the model has not been used to test psychological interventions in GAD.

Inhalation of 7.5% CO_2 has been shown to be associated with a range of neuro-psychological biases in attention and emotion processing characterising clinical anxiety[122]: healthy subjects exposed to the 7.5% CO_2 model showed significantly reduced accuracy rates on the anti-saccade task towards threat-related picture cues[143], this is consistent with findings in HA vs. LA healthy subjects exposed to threat-related pictures[124], and with the finding that patients with GAD show enhanced orientation towards threat-related stimuli in a modified probe detection task[158]. Healthy subjects exposed to the 7.5% CO_2 model showed significantly enhanced alerting and orienting attentional network performance (hypervigilance) on the ANT[127], as well as increased distractibility and impaired attention control to threat-cues[127]. The 7.5% CO_2 challenge is therefore considered a potential novel experimental model of subjective, autonomic and neuropsychological features of generalized anxiety in humans that can be useful in the early-phase evaluation of therapeutic interventions for GAD.

7.1.2. Threat-attention in anxiety

Anxiety is associated with distinct attentional patterns – these include reduced attentional control in the presence of negatively valenced and threatening stimuli[328], hypervigilance to threatening and negatively valenced stimuli[329], increased distractibility[328,330], delayed disengagement from threat-related stimuli, and a bias towards interpreting emotionally ambiguous stimuli as threatening[9]. The attention control theory of anxiety describes a disruption of attentional resource-allocation

balance, from 'top-down' (goal directed) towards 'bottom-up' (stimulus driven) attention[331]. These patterns may be associated with dysfunctional attention-control mechanisms underpinning pathological phenomena such as worry, anxiety and rumination.

Attentional control and attentional bias in anxiety have been explored in both clinical and non-clinical populations using a range of experimental tasks (see chapter 4 section 4.1.1. for a detailed discussion). Meta-analyses have found evidence for enhanced vigilance for threat, and for more difficult threat-disengagement[116]; as well as a consistent attentional bias towards conscious and non-conscious threat-related stimuli across a range of experimental paradigms and conditions, across clinical diagnostic categories, different age groups, and extended to non-clinical high anxiety subjects but not to non-anxious subjects [117].

7.1.3. tDCS

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation modality, which changes cortical tissue 'excitability' as a result of applying a weak (0.5– 2 mA) direct current via scalp electrodes overlying targeted cortical areas[253]. In contrast to other neuro-stimulation modalities, tDCS does not directly trigger action potentials in neuronal cells, but instead changes overall tissue excitability, and therefore may be more aptly regarded as a 'neuro-modulatory' rather than a neuro-stimulatory approach[232]. Cortical tissue underlying the anode (positive electrode) becomes hypo-polarized, and therefore hyper-excitable; areas underlying the cathode (negative electrode) become less excitable as the average resting potential becomes more

polarized. The magnitude of these membrane polarisation changes is not in itself sufficient to directly cause neurons to fire[233]. These effects continue after electrical stimulation ceases, and a single application may be associated with tissue excitability changes lasting up to a few hours[234,235,380]. These findings suggest tDCS is likely to be associated not only with transient membrane polarisation changes, but also with longer-lasting synaptic changes[296]. The mechanisms facilitating these tDCS after-effects may involve glutamatergic synapses and intra-cortical inter-neurons[90,381].

7.1.3.1. tDCS and attention to threat

A single session of active vs. sham left-DLPFC tDCS has been shown to improve attentional bias acquisition in a targeted direction, when delivered concurrently with computerised attention bias modification training (ABMT)[400]. A recent study assessed the effect of a single session of DLPFC tDCS on emotional processing, using the dot-probe task[418]. The study compared active vs. sham tDCS, utilising two commonly-used tDCS montages: bipolar balanced (anode over F3 in the 10/20 system of electrode placement, cathode over F4), and bipolar unbalanced (anode over F3, cathode over F8). Sixty healthy participants were randomised to receive a single session of active bipolar balanced, active bipolar unbalanced, or sham tDCS, before undertaking a dot-probe measure of vigilance to threat. The study found that participants in the bipolar-balanced (but not in the bipolar unbalanced) group demonstrated reduced vigilance to threat compared to the sham tDCS group. No significant effects of tDCS on other measures of emotional processing were found.

7.1.3.2. tDCS effects on anxiety

In contrast to the growing evidence base for the use of transcranial direct current stimulation in depression, there is relatively little published research about its use in anxiety disorders[298]. To date, there are no published randomised controlled trials of tDCS in anxiety disorders. There is a limited number of published case studies describing the treatment of patients suffering from a range of anxiety disorders with tDCS (see Chapter 2).

7.1.3.3.   tDCS effects on cognitive and emotional processing

There have been only few studies of the effects of tDCS on attention. A study in patients with neurological neglect demonstrated that anodal tDCS at 1 mA over the affected PPC, and cathodal tDCS over the unaffected PPC, was associated with overcoming the ipsilateral line bisection bias[382]. Findings from pre-clinical studies of the effects of tDCS on attention include the following:

1. anodal tDCS at 1mA over the posterior parietal cortex (PPC) changed the direction of attention towards the contralateral hemi-space, whereas cathodal stimulation had the opposite effect[382].

2. cathodal tDCS at 2.0 mA over the right intra-parietal sulcus (IPS) increased top-down control in a visual attention task[383].

3. anodal tDCS at 1.0mA over left dorsolateral prefrontal cortex (L-DLPFC) improved vigilance decrement in a simulated air traffic controller task[384].

4. Active vs. sham anodal tDCS at 2.0mA over right inferior frontal cortex (F10) was associated with improved object detection and ANT alerting network performance[247].

5. Anodal tDCS over right PPC was associated with faster attentional orienting responses to contralateral (but not to ipsilateral) targets[385].

6. Anodal (vs. cathodal) tDCS at 1.5mA over the pre-supplementary motor area was associated with increased inhibitory control in a stop signal task[386].

7. tDCS at 1 mA over Dorsolateral Prefrontal Cortex is associated with improved performance on the Tower of London task[387].

8. Roy and colleagues recently found that tDCS at 1.5 mA over the right parietal cortex (but not the left parietal cortex or left dorsolateral prefrontal cortex) was associated with improving orienting network performance on the ANT.

9. Right parietal cortex tDCS also selectively improved mean network efficiency for targets presented in the left (contralateral) visual field[90].

10. The study described in chapter 6 demonstrated a significant superiority of executive control (but not orienting or alerting) network performance following

active vs. sham tDCS over the left DLPFC.

7.1.3.4.   The Anti-saccade task

The anti-saccade task (Figure 4.1), first described by Haslett [118] has emerged as an important tool for investigating attention control (see chapter 1 and chapter 4 for a fuller discussion of the anti-saccade task). The task requires a two-step process: 1. suppression of the automatic pro-saccade (the tendency to look towards the target), and 2. generating a voluntary anti-saccade towards the position mirroring the presented target. The task decouples stimulus location from saccade goal, and requires inversion of the stimulus vector to generate the saccade vector. Correct performance on the anti-saccade task requires top-down control to prevent express-saccade related

directional errors. The anti-saccade task provides two performance measures[122]:

1. *Performance effectiveness* – anti-saccade accuracy rate;

2. *Performance efficiency* – correct saccade latency.

Reduced anti-saccade accuracy may reflect impairment of frontally mediated inhibitory control[419,420], and may be associated with impaired working memory, and attentional focus[421-423]. Compared to low-anxious (LA) subjects, high anxious (HA) subjects have longer correct anti-saccade (but not pro-saccade) latencies, suggesting that the anxiety-related deficit is in the inhibitory component of attentional control, leading to reduced efficiency; however there were no significant differences between HA and LA subjects in error rate, suggesting that anxiety reduces efficiency but not effectiveness [75,123]. Subjecting healthy participants to severe threatening-stimuli version of the anti-saccade task (using aversive images from the International Affective Picture Set) resulted in an elevated error rate (reduced effectiveness) in HA vs. LA participants[124] which may reflect the additional cognitive processing required to over-ride the attentional bias towards threatening stimuli in HA participants. Use of the 7.5% CO_2 paradigm to induce anxiety in healthy individuals is associated with longer anti-saccade latencies (reduced efficiency) and with increased anti-saccade errors towards threat-related stimuli (reduced effectiveness)[127]. A study of evoked response potentials (ERPs) during pro-saccade and anti-saccade tasks found that compared to LA individuals, HA individuals had longer anti-saccade latencies, and lower ERP activity, at frontocentral and central recording sites, immediately prior to correct anti-saccade trials. The authors concluded that this provided evidence of anxiety-related reduced recruitment of frontal top-down attentional control resources needed for suppression of reflexive

pro-saccade[128].

To date there has been only one study published describing the effects of tDCS on anti-saccade task performance[248]. The study examined tDCS modulation of frontal eye field (FEF) excitability. The study found that on pro-saccade trials, anodal (but not cathodal) tDCS shortened the latency of saccades to a contralateral visual cue; in anti-saccade trials, cathodal (but not anodal) tDCS increased ipsilateral latency. Anodal tDCS increased saccade accuracy toward contralateral visual cues.

7.2. Aims and predictions

This study compares the effects of a single session of active vs. sham anodal tDCS over the left DLPFC on attention control as measured using the anti-saccade task while healthy participants undertook the 7.5% CO_2 challenge – a novel experimental model of human anxiety. Participants undertook a 20 minute 7.5% CO_2 challenge, in the midst of which they competed a modified (emotional) anti-saccade task. By using the 7.5% CO_2 challenge, this study progresses further towards testing the utility of tDCS as a treatment for anxiety. Employing a similar design to the study described in chapter 5, allows for comparison of effects between mindfulness and tDCS.

We predicted :

- That compared to the sham tDCS, active DLPFC tDCS would be associated with reduced threat-related attentional biases on the anti-saccade task (increased

anti-saccade accuracy and reduced correct-anti-saccade latency), and with reduced self-report anxiety, but not with reduced autonomic arousal.

- That the effect of active vs. sham tDCS on anti-saccade accuracy and on correct-saccade latency would be greater with negatively vs. neutrally-valenced cues.

- That the 7.5% CO_2 challenge would be associated with significant increases in autonomic arousal and self-report anxiety.

7.3. Method

A between-subjects design comparing the effects of active vs. sham tDCS during a 7.5% CO_2 challenge, on anti-saccade task performance, and on autonomic arousal and self-report anxiety. An effect size of ($\eta 2P \geq 0.1$) was predicted, based on the study described in chapter 5. An a-priori power calculation for a repeated measures ANOVA with between-within interactions with 3 variables (group x trial type x valence), revealed that a sample size of n=20 (10 vs. 10) was required in order to detect an effect size of ($\eta 2P = 0.1$) with an alpha level of 0.05 and high power of 0.8.

7.3.1. Participants

36 healthy volunteers (mean age = 21.37 years, SD = 2.9) (22 women and 14 men), were recruited, having responded to advertised offers of course credit / money (£20) for taking part in the study. Exclusion criteria were: history of epilepsy, hypertension (above 140/90mmHg), or mental illness (including depression and/or anxiety), the presence of metal implants (including pacemakers, dental implants etc.), current self-reported pregnancy or breastfeeding, weekly alcohol intake exceeding 50 units for males or 35 units for females. Candidates who had over the previous 8 weeks taken

medication other than topical preparations, oral contraceptives, paracetamol, or aspirin, or whose body mass index (BMI) was outside the range of 18-28, were also excluded. Participants were initially screened using a telephone health screening tool. They were further screened on the day of the study session using the Mini International Neuropsychiatric Interview (MINI)[372] – this structured clinical interview is widely used to screen healthy volunteers for mental disorders using DSM IV[390] diagnostic criteria. Participants were randomised to one of two experimental groups in a double-blind, gender balanced design (figure 7.1): active tDCS (n=18) and sham tDCS (n=18)
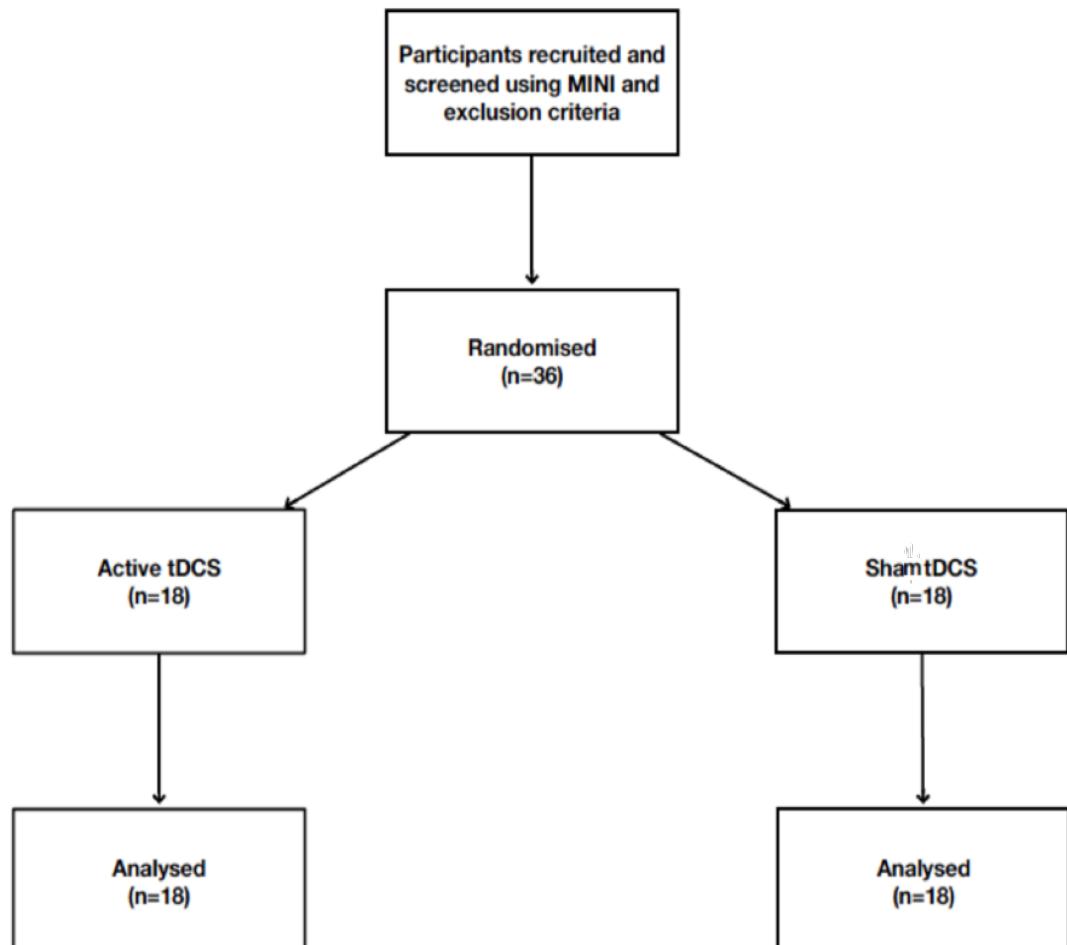
Figure 7.1: Consort diagram showing study design and progress of subjects through the study.

7.3.2. Study design and workflow

This study was approved by the research ethics and governance committee at the University of Southampton (See figure 7.2 for study workflow). Participants were recruited using advertisements, and underwent telephone-screening, before being invited for a single, 3-hour test session. Participants were instructed to abstain from alcohol and caffeine in the 24 hours before attending the test session. On arrival, participants were briefed, and provided informed consent. They were then screened using the MINI and also underwent blood-pressure and heart-rate screening. Participants completed a set of baseline trait and state measures (STAI, ASI, GAD-7, ACS, PSWQ, SSAI, PANAS, and VAS) (described in previous chapters), as well as having baseline measures of blood pressure and heart rate taken. Participants underwent 20 minutes of active/sham tDCS. Immediately after tDCS, another set of self report measures (subjective anxiety and mood) and blood-pressure and heart rate was recorded. Participants then underwent a 20-minute, 7.5% CO_2 challenge. 10 minutes into the challenge, participants began the anti-saccade task. Immediately after the 7.5% CO_2 challenge, participants completed another set of self-report measures including the ACS and the PSWQ. Participants left following debriefing session, and were contacted 24 hours later to discuss concerns and report any adverse effects.
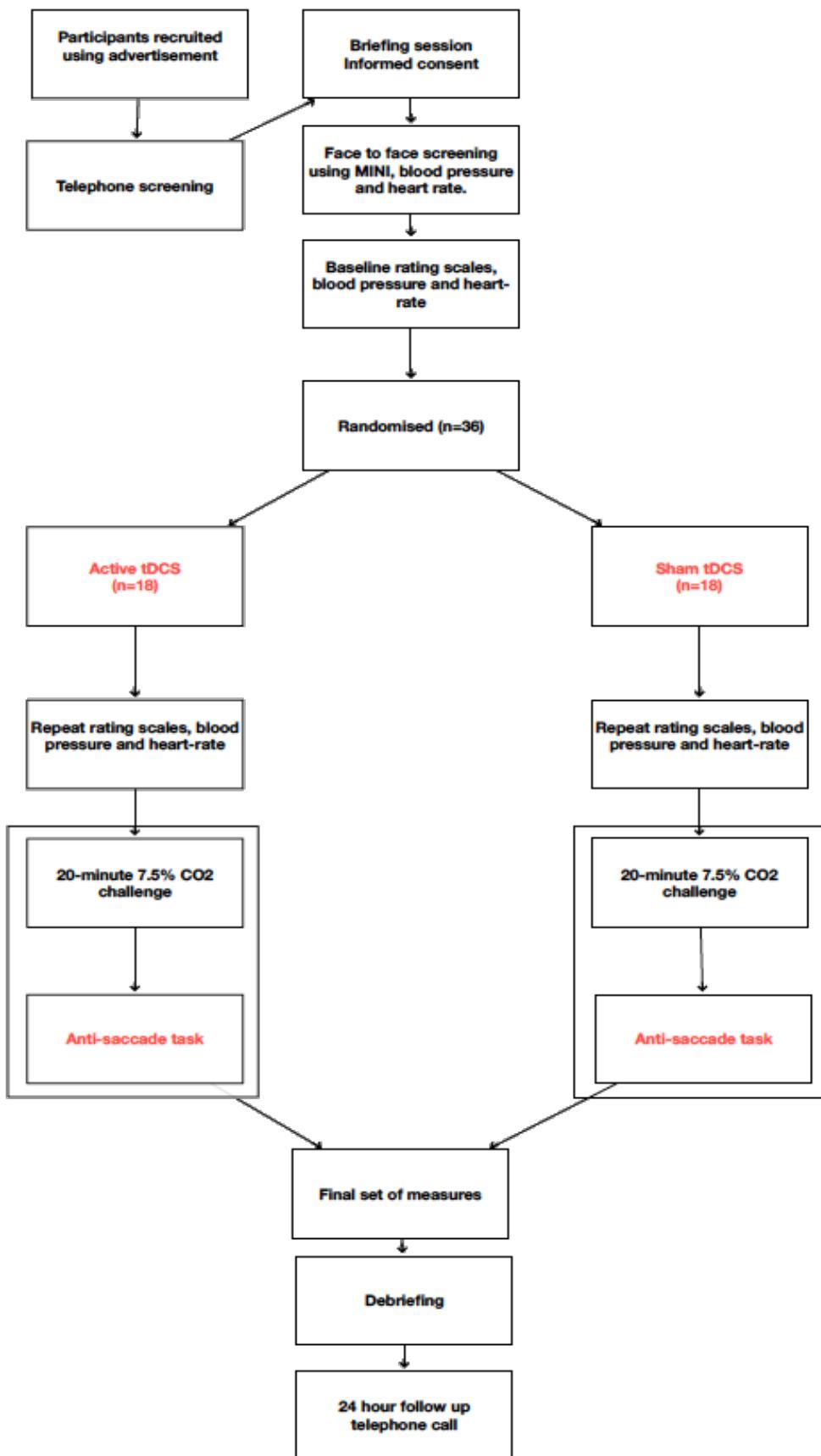
Figure7.2: Procedural diagram describing Study design and workflow

7.3.3. Experimental interventions

7.3.3.1. tDCS

The tDCS montage utilised was bi-frontal, with the anode placed over the L-DLPFC and the cathode over the R-DLPFC. This is the commonly used montage in modern tDCS trials in depression. Two tDCS stimulators were used; these were coded using numbered labels and pre-programmed to deliver either active or sham tDCS. Active tDCS was delivered using a 2mA current setting for 20 minutes. Sham tDCS was delivered using a brief (15 second) ramp-up, ramp-down protocol to simulate active stimulation and aid in blinding. Participants and administering staff were blinded to active/sham tDCS allocation. The tDCS apparatus used in this study was the Magstim HDCkit (Handheld Direct Current). HDCkit consists of a programming unit, stimulator, and two electrodes (4 X 4cm). The electrodes are covered in a sponge envelope soaked in normal saline (0.9% NaCl).

7.3.3.2. 7.5% CO_2 challenge

Participants used an oro-nasal face mask to breath a mixture of: CO_2 7.5%, O2 21%, and N2 71.5%. The session lasted 20 minutes. 10 minutes into the session, participants undertook a modified (emotional) anti-saccade task (see section 7.2.4.1. below).

7.3.4. Outcome measures

7.3.4.1. Primary outcome measure

7.3.4.1.1. Anti-saccade task:

This study used the modified (emotional) version of the anti-saccade eye-movement task (see Chapter 1 and chapter 4 for a more detailed discussion of the anti-saccade task). The emotional cues used in the task were 8 negative and 8 neutral pictures selected from the standardized International Affective Picture Set[143,354]. Participants completed 96 randomly-ordered trials (24 trials per saccade-type x picture valence condition). Trials were counter-balanced for stimulus location. The task was presented using Inquisit 2 computer software. Horizontal eye-movements were measured by electro-oculography and sampled at 1000 Hz (MP150-amplifier and AcqKnowledge 3.8.1 software, Biopac systems, Goleta, CA).

The task requires a two-step process: 1. suppression of the automatic pro-saccade (the tendency to look towards the target), and 2. generating a voluntary anti-saccade towards the position mirroring the presented target. Correct performance on the anti-saccade task requires top-down control to prevent express-saccade related directional errors. The anti-saccade task provides measures of attention control (pro-saccade inhibition) and selective attention (comparative accuracy and correct saccade-latency when presented with negative vs. neutral affective cues). The task provides two performance measures[122]:

1. Performance *effectiveness* – anti-saccade accuracy rate;

2. Performance *efficiency* – correct saccade latency.

Compared to low-anxious (LA) subjects, high anxious (HA) subjects have longer correct anti-saccade (but not pro-saccade) latencies – suggesting that the anxiety-related deficit is in the inhibitory component of attentional control, leading to reduced efficiency; there were no significant differences between HA and LA subjects in error

rate – investigations suggesting that anxiety reduces efficiency but not effectiveness [75,123]. Subjecting healthy participants to severe threatening-stimuli version of the anti-saccade task (using aversive images from the International Affective Picture Set) resulted in an elevated error rate (reduced effectiveness) in HA vs. LA participants[124] – this may reflect the additional cognitive processing required to over-ride the attentional bias towards threatening stimuli in HA participants. Saccade-data was pre-analysed similarly to the methodology described in Chapter 4. The data were scored by a blinded assessor.
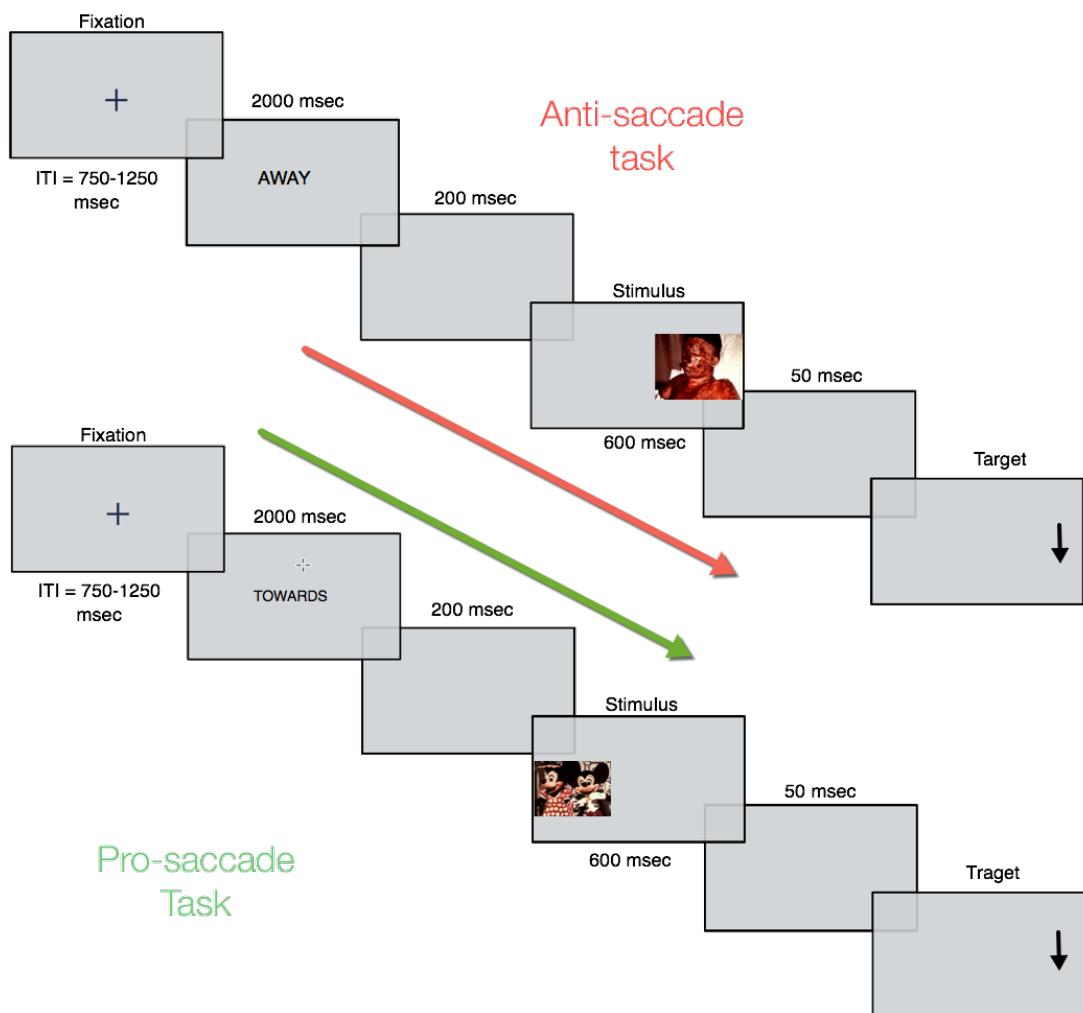
Figure 7.3: The anti-saccade task

7.3.4.2.   Secondary outcome measures:

7.3.4.2.1.   Spielberger State-Trait Anxiety Inventory[335]

The Spielberger State-Trait Anxiety Inventory is a widely accepted instrument for measuring both state and trait anxiety. It has been translated into more than thirty languages, and cited in over 3000 studies[313]. The Spielberger State-Trait Anxiety Inventory consists of 40 items, 20 aimed at assessing trait anxiety (Spielberger Trait Anxiety Inventory - STAI), and 20 measuring state anxiety (Spielberger State Anxiety Inventory - SSAI). Each item is a 4 point forced-choice Likert scale[314]. The state and trait scales each contain 2 factors: "anxiety absent" and "anxiety present". Trait anxiety is a tendency towards feeling anxiety, worry, discomfort, and stress; it describes a relatively stable characteristic or disposition, rather than a reaction to a particular set of conditions. The STAI addresses how subjects feel generally, commonly or usually. Items on the trait scale include statements such as: "I am content", "I have disturbing thoughts". Each item on the trait scale is scored: 1 almost never; 2 sometimes; 3 often; 4 almost always. State anxiety is a transient response to a set of conditions perceived as threatening or dangerous. It can include components such as autonomic arousal, and feelings of fear, discomfort, or nervousness. The SSAI measures how subjects feel "right now, at this moment"[314]. Items on the state scale include statements such as: "I am calm", "I am worried". Each item on the state scale is scored: 1 not at all; 2 somewhat; 3 moderately so; 4 very much so. Scores generated on each scale can range from 20 – 80, higher scores reflect more intense anxiety[314]. The Spielberger State-Trait Anxiety Inventory was found to have good internal consistency (average αs > 0.89)[314]. The test-retest reliability at different time points of the trait scale (STAI) is also good

(average r = 0.88)[315]. The test-retest reliability of the state scale (SSAI) at different time-points is of course lower (r = 0.70).

7.3.4.2.2. Visual Analogue Scale (VAS)

This can be used to measure a construct across a continuum of values, when direct measurement is difficult or impossible to achieve. Examples of characteristics that can be measured using a VAS include subjective pain, intensity of emotional experience, or attitudinal response. It is important for the construct to be perceived as a continuum rather than a set of discrete steps or values. The VAS is presented as a 100mm horizontal line, stretching between two anchor statements defining the end points of a continuum, with optional additional statements situated next to the line, indicating waypoints along the continuum. The subject is tasked with indicating their position along the continuum by marking the line. Measuring the distance in millimeters from the left end of the line to the marked point derives the score. In this study, we presented subjects with 6 VAS scales items relating to subjective feeling: 1 "alert"; 2 "worried"; 3 "happy"; 4 "relaxed"; 5 "anxious"; 6 "feel like leaving". Each was anchored by 2 statements: from left:" Not at all", to right: "All the time". The 6 scales make up 3 groups: cognition (1), positive affect (3, 4), and negative affect (2, 5, and 6). VAS scales are widely used for measuring pain and other psychological states, and have also been validated in anxiety[357]. The evidence suggests that VAS scales perform well in comparison to subjective measures such as the Likert and Borg scales[358,359].

7.3.4.2.3.   GAD-7[391]

The GAD-7 is a 7 item questionnaire validated and extensively used for screening and assessing the severity of Generalised Anxiety Disorder (GAD) in clinical practice and research[391]. The scale prompts subjects to respond to the question "Over the last 2 weeks, how often have you been bothered by the following problems?: 1 Feeling nervous, anxious or on edge; 2. Not being able to stop or control worrying; 3. Worrying too much about different things; 4. Trouble relaxing; 5. Being so restless that it is hard to sit still; 6. Becoming easily annoyed or irritable; 7. Feeling afraid as if something awful might happen". Each item is scored 0-3 on a Likert-like scale offering the following response choices: 0:"Not at all"; 1: "Several days"; 2: "More than half the days"; 3: "Nearly every day". Anxiety severity cutoffs used are : Mild anxiety ≥5, Moderate anxiety ≥ 10, Severe anxiety ≥ 15. A GAD-7 score of ≥ 10 has sensitivity of 89% and specificity of 82% for GAD[392]. In this study, a modified the GAD-7 was used to assess the severity and change of anxiety comparing pre and post tDCS stimulation scores. Participants were asked to rate their experience over "the last 20 minutes" rather than over the last 2 weeks as in the original GAD-7. A Visual Analogue Scale (VAS) was used, anchored from left "Not at all" to right "All the time".

7.3.4.2.4.   The Penn State Worry Questionnaire (PSWQ)[337]

The PSWQ is a well-established self-report instrument used to measure subjects' worry trait. The PSWQ contains 16 items relating to worry; each item is scored on a 5 point Likert scale ranging from 1 "Not at all typical of me" to 5 "Very typical of me". Eleven

items are positively scored – for example: "I am always worrying about something" ,

and 5 items are scored in reverse – for example: "I do not tend to worry about things".

Higher scores are correlated with increased tendency to worry. PSWQ demonstrates

good internal consistency (α = 0.90)[340], and test-retest reliability[341].

7.3.4.2.5.   Anxiety Sensitivity Index (ASI)[393]

Anxiety sensitivity is a concept describing the fear of physiological, psychological, or

behavioural manifestations of anxiety ("fear of fear"), based on beliefs that these

sensations predict adverse physical, psychological, or social consequences[394]. Anxiety

sensitivity is seen as possessing a cognitive dimension rooted in dysfunctional beliefs

about the meaning of anxiety manifestations, and therefore goes beyond simple

Pavlovian conditioning arising out of aversive anxiety experiences. Anxiety sensitivity is

seen as preceding (and possibly even predicting) the onset of anxiety disorders[395]. The

ASI is one of the most widely used instruments measuring anxiety sensitivity in clinical

and non clinical populations[396]. The ASI is an 18 item questionnaire comprised of three

6 item subscales relating to physical, cognitive and social concerns. The ASI

demonstrated good internal consistency and test-retest reliability (p=0.72; r =

0.75)[393,396,397]. In this study the ASI was utilised to assess potential interaction between

subjects' somatic anxiety sensitivity and reported tDCS adverse effects.

7.3.4.2.6.   Positive and Negative Affect Scale[374]

The PANAS is a 20 item questionnaire assessing affect along a 5-point Likert scale

ranging from 1 "Very slightly or not at all" to 5 "Extremely". The PANAS is composed of

2 10-item sub-scales: Positive (PANAS-P) and Negative (PANAS-N). Both sub-scales

demonstrate good internal consistency: PANAS-P α = 0.89, PANAS-N α = 0.85. Each

item introduces an emotion (e.g. excited, enthusiastic, ashamed, hostile) and invites

the subject to rate the extent to which they feel this emotion. Higher scores on the

PANAS indicate more positive affect. This study used a PANAS state version, asking

subjects to rate their emotions "in the past 20 minutes".

7.3.4.2.7. Attentional Control Scale (ACS)[114]

The ACS is a 20-item self-report measure assessing the ability to maintain attentional

control in the presence of distractors such as environmental stimuli, concurrent tasks,

and emotional states. The ACS presents items such as "When I am reading or studying,

I am easily distracted if there are people talking in the same room", and "When trying

to focus my attention on something, I have difficulty blocking out distracting thoughts";

it then asks subjects to rate themselves along a 4 point Likert frequency of experience

scale ranging from 1 "Almost never" to 4 "Always". The ACS demonstrates good

internal consistency using a two factors Focusing (α= 0.82) and Shifting (α = 0.71) sub-

scales[342].

7.4. Results

7.4.1. Participants

The active and sham tDCS groups did not significantly differ in gender [t=0.669; *p* =

0.51) or age (t=147; *p* = 0.88). One-way ANOVAs confirmed that there were no

statistically significant differences between groups in their baseline self-reported

measures (see table 7.1). Levels of anxiety in the cohort accorded with expected levels

in healthy volunteers[343]; and dispositional mindfulness levels on the MAAS were in

keeping with normative values for young adults[344]. All self-report measures

demonstrated good internal consistency (a's > 0.74).

Table 7.1: Comparison of participant demographics and characteristics at study entry for active and sham tDCS groups

	Active tDCS		Sham tDCS			
	n=18		n=18			
	M	SD	M	SD	t(34)	*p*
Gender (F:M)	12:6		10:8			
Age (years)	21.4	2.9	21.3	3.2	0.15	*p=0.88*
BMI (kg/m²)	21.9	3.0	22.6	2.5	-0.73	*p=0.47*
STAI-T	31.2	5.0	34.5	6.7	-1.64	*p=0.11*
GAD-7	21.3	10.6	18.1	10.4	0.92	*p=0.37*
ASI	38.0	10.3	38.9	10.7	-0.27	*p=0.79*
ACS	50.8	7.1	49.3	9.5	0.52	*p=0.61*
PSWQ	38.2	13.4	37.1	12.1	0.27	*p=0.79*

BMI = Body Mass Index; STAI-T = Spielberger State-Trait Anxiety Inventory – Trait sub-scale; ASI = Anxiety Sensitivity Index; ACS = Attentional Control Scale; PSWQ = Penn State Worry Questionnaire

7.4.2. Effects of active vs. sham tDCS on attention control as measured on the anti-saccade task during 7.5% CO_2 challenge

7.4.2.1. Saccade accuracy:

Saccade error-rates for active vs. sham tDCS groups across cue-valence, and trial type (pro vs. anti-saccade) are shown in table 7.2. A 2 (Group: active vs. sham tDCS) X 2

(trial-type: anti vs. pro-saccade) x 2 (Cue-valence: negative vs. neutral) ANOVA demonstrated:

- A significant main effect of trial-type [F(1,34)=173.71; $p < 0.001$, $\eta^2_P = 0.84$]: pro-saccade error rates (Mean = 4%) across groups were significantly lower than anti-saccade error rates (Mean = 59%).

- A significant interaction Cue-valence x Group [F(1,34) = 4.06; $p = 0.05$, $\eta^2_P = 0.11$]: In the active tDCS group, the error rates in negatively-cued (pro- and anti-saccade) trials were lower than in neutrally-cued trials ($p = 0.04$); there was no significant difference between error rates in negatively vs. neutrally-cued trials in the sham-tDCS group.

No other significant main effects or interactions were found (all F's (2,34) < 2.55, p's > 0.12).

These results indicate that as would be expected in the anti-saccade task, error rates across groups were lower in pro-saccade trials than in anti-saccade trials. Active tDCS was also associated with significantly lower error-rates in negatively-cued compared to neutrally-cued trials, which may reflect reduced attention to threat.

7.4.2.2. Correct-saccade latency

Correct-saccade latency for active vs. sham tDCS groups across cue-valence, and trial type (pro vs. anti-saccade) are shown in table 7.2. A 2 (Group : active vs. sham tDCS) X 2 (trial-type: anti vs. pro-saccade) x 2 (Cue-valence: negative vs. neutral) ANOVA demonstrated:

- Significant main effect of Trial-type [$F_{(1,34)}$ = 235.23; $p < 0.001$, η^2_P = 0.89]: pro-saccade latencies (Mean = 168.81 msec) were shorter than anti-saccade latencies (Mean = 276.36 msec).

No other significant main effects or interactions were found (all F's < 1.40, p's > 0.25). Independent samples t-test showed a trend for longer pro-saccade latencies towards negative cues in the active vs. sham tDCS (t(34) = 1.94, p = 0.06, table 7.2).

Table 7.2: Group x cue-valence x trial-type mean error rates and correct saccade latency.

	Active tDCS		*Sham tDCS*		*t(34)*		*p*	
	n=18		n=18					
Cue-valence	Neg	Neut	Neg	Neut	Neg	Neut	Neg	Neut
Pro-saccade error-rate	0.028 (0.06)	0.041 (0.05)	0.037 (0.04)	0.039 (0.06)	*-0.53*	*0.13*	*0.60*	*0.90*
Anti-saccade error-rate	0.556 (0.25)	0.622 (0.25)	0.590 (0.23)	0.576 (0.25)	*-0.44*	*0.55*	*0.67*	*0.59*
Correct pro-saccade latency	175.02 (29.63)	173.50 (22.23)	159.18 (15.84)	166.1 (17.48)	*1.94*	*1.08*	*0.06*	*0.29*
Correct anti-saccade latency	298.39 (63.41)	270.41 (57.19)	278.84 (51.57)	*272.81 (52.40)*	*0.96*	*-0.12*	*0.35*	*0.91*

7.4.3. Effects of active vs. sham tDCS on self-report anxiety and affect

A mixed-design 2 (Group: active vs. sham tDCS, between subjects) x 3 (Time: Baseline vs. post-tDCS vs. post CO_2 challenge – within subjects) ANOVA of self-report measures of anxiety and affect found:

- No significant main effect of Group (all F's < 0.94, p's > 0.34)

- No significant interaction Group x Time (all F's < 1.27, p's > 0.29)

- Main effect of Time on all but one self-report anxiety and affect measures (table 7.3) – these reflect increased anxiety, increased negative affect, and decreased positive affect following the 7.5% CO_2 challenge

Table 7.3: Effects of Time (Baseline vs. post-tDCS vs. post CO$_2$ challenge) on self-report measures of anxiety and affect

		Active tDCS n=18		Sham tDCS n=18			
		Mean	SD	Mean	SD	t(34)	p
GAD7	Baseline	13.64[a]	12.43	14.32[a]	9.28	-0.19,	p=.85
	Post-tDCS	18.33[a]	18.24	11.90[a]	10.43	1.30,	p=.20
	Post CO$_2$	41.07[b]	25.69	41.92[b]	24.30	-0.10,	p=.92
SSAI	Baseline	29.89[a]	7.57	33.00[a]	7.82	-1.20,	p=.24
	Post-tDCS	32.06[a]	10.12	32.47[a]	8.57	-0.13,	p=.90
	Post CO$_2$	49.94[b]	2.33	51.00[b]	2.57	-0.28,	p=.21
PANAS positive	Baseline	31.17[a]	7.02	31.61[a]	7.41	-0.19,	p=.86
	Post-tDCS	29.12[b]	8.02	12.17[a]	8.93	0.16,	p=.88
	Post CO$_2$	23.41[c]	8.20	11.50[a]	7.65	0.42,	p=.68
PANAS negative	Baseline	12.44[a]	4.26	20.28[b]	2.30	0.24	p=.81
	Post-tDCS	11.78[a]	2.76	28.65[a]	1.98	0.35	p=.73
	Post CO$_2$	17.47[b]	7.30	21.80[a]	7.39	-1.13	p=.27
VAS negative	Baseline	23.20[a]	19.63	79.54[b]	17.56	-0.88	p=.39
	Post-tDCS	26.06[a]	21.44	69.11[a]	21.52	0.60	p=.56
	Post CO$_2$	75.00[b]	20.03	64.64[ab]	19.21	0.16	p=.88
VAS positive	Baseline	67.47[a]	14.12	56.33[b]	13.67	-0.69	p=.49
	Post-tDCS	66.58[ab]	12.59	58.00[a]	13.81	0.44	p=.66
	Post CO$_2$	63.25[b]	29.62	49.14[b]	18.32	0.84	p=.41
VAS cognition	Baseline	49.94[a]	20.26	71.56[c]	25.70	-1.04	p=.30
	Post-tDCS	46.97[b]	21.70	12.17[a]	30.24	-0.25	p=.81
	Post CO$_2$	65.19[c]	36.73	11.50[a]	27.70	-0.59	p=.56

SSAI = Spielberger State Anxiety Inventory; PANAS = Positive and Negative Affect Scale; VAS = Visual Analogue Scale. Within each measure and group values with different superscripts significantly differ, *p* < 0.05 .

7.4.4. Effects of active vs. sham tDCS on measures of autonomic arousal

See table 7.4 for effects of time on measures of autonomic arousal. A 2 (Group : active vs. sham tDCS; between subjects) x 3 (Time: baseline vs. post-tDCS vs. post- CO_2; within subjects) repeated measures **ANOVA of heart rate** showed:

- A significant main effect of Time [$F_{(1,34)}$ = 12.46, p < 0.0005, η^2_P=0.26], reflecting the increase in heart-rate post-CO_2 challenge as compared to baseline and to post-tDCS.

- No significant main effect of Group (active vs. sham tDCS), and no significant Time x Group interaction [$F's_{(1,34)}$ < 1.00, p > 0.37].

A 2 (Group : active vs. sham tDCS; between subjects) x 3 (Time: baseline vs. post-tDCS vs. post- CO_2; within subjects) repeated measures **ANOVA of systolic blood pressure** showed:

- A significant main effect of Time [$F_{(1,34)}$ = 7.74, p = 0.05, η^2_P=0.276], reflecting the increase in systolic blood pressure post-CO_2 challenge as compared to baseline and to post-tDCS.

- No significant main effect of Group (active vs. sham tDCS), and no significant Time x Group interaction [$F's_{(1,34)}$ = 0.123, p > 0.88].

A 2 (Group : active vs. sham tDCS; between subjects) x 3 (Time: baseline vs. post-tDCS vs. post- CO_2; within subjects) repeated measures **ANOVA of diastolic blood pressure** showed:

- A significant main effect of Time [$F_{(1,34)}$ = 3.82, p = 0.03, η^2_P=0.10], reflecting a significant increase in diastolic blood pressure from post-tDCS to post- CO_2.

- No significant main effect of Group (active vs. sham tDCS), and no significant Time x Group interaction [F's$_{(1,34)}$ < 2.41, p > 0.13].

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Table 7.4: Effects of Time (Baseline vs. post-tDCS vs. post CO_2 challenge) on measures of autonomic arousal

		Active tDCS		Sham tDCS			
		n=18		n=18			
		Mean	SD	Mean	SD	t(34)	p
HR	Baseline	72.22[a]	12.62	72.78[a]	9.91	0.15,	p= .88
	Post-tDCS	69.00[a]	20.33	71.11[a]	9.20	0.59,	p=.56
	Post CO_2	80.20[b]	15.91	77.72[b]	14.54	0.48,	p=.63
Systolic BP	Baseline	117.94[a]	12.83	122.06[a]	11.96	0.99,	p=.33
	Post-tDCS	108.11[a]	29.93	116.67[a]	12.24	1.12,	p=.27
	Post CO_2	121.94[b]	13.54	129.94[b]	15.05	1.47,	p=.15
Diastolic BP	Baseline	70.94[a]	17.06	71.17[a]	7.06	0.05,	p=.96
	Post-tDCS	61.00[b]	22.02	69.94[a]	10.11	1.57,	p=.13
	Post CO_2	71.94[a]	9.97	73.00[a]	8.13	0.35,	p=.73

7.4.5. Adverse outcomes and side effects

No adverse outcomes or side effects were reported during this study.

7.4.6. Blinding integrity

14 of the 18 participants (77.8%) in the active tDCS group correctly guessed their group allocation. In the sham tDCS group 11 out of 18 (61.1%) correctly guessed their allocation.

7.5. Discussion

This study examined the effects of a single, 20-minute session of active vs. sham anodal tDCS over the left DLPFC on attention control and threat-related attentional bias, as

measured by the anti-saccade task, in healthy participants undergoing the 7.5% CO_2 challenge – a novel model of human generalised anxiety.

7.5.1. Anti-saccade task

We predicted that compared to the sham tDCS, active DLPFC tDCS would be associated with reduced threat-related attentional biases on the anti-saccade task (increased anti-saccade accuracy and reduced correct-anti-saccade latency), and that these effects would be more marked in anti-saccades cued by threat-related (compared to neutral) pictures.

7.5.1.1. Saccade accuracy

Both groups showed the expected lower error rates in pro-saccade compared to anti-saccade trials. This is an inherent feature of the anti-saccade task, because pro-saccades are reflexive, whereas anti-saccades require reflex-inhibition, followed by initiation of a saccade in the opposite direction.

The results showed that a single 20-minute session of active anodal tDCS (2mA) over left DLPFC was associated with increased accuracy in threat-cued saccade trials (across pro- and anti-saccade trials); this difference was not found in the sham tDCS group. There was also a trend towards active tDCS to be associated with higher accuracy in threat-cued anti-saccades vs. neutrally-cued anti-saccades. There were no significant within- or between-group differences in pro-saccade accuracy – reflecting a ceiling effect in pro-saccade accuracy (> 95%).

7.5.1.2. Correct-saccade latency

Both active and sham tDCS groups showed the expected shorter latencies in pro-saccade vs. anti-saccade trials. This is an inherent feature of the anti-saccade task, because pro-saccades are reflexive, whereas anti-saccades require reflex-inhibition, followed by initiation of a saccade in the opposite direction. There was also a trend towards the active tDCS group to have longer latency on threat-cued, pro-saccade trials ($p = 0.06$), as compared with the sham tDCS group – although we predicted that active vs. sham tDCS would be associated with faster disengagement from threat-related stimuli, these results fit a pattern of slower engagement with threat-related stimuli.

Collectively, the accuracy analysis indicates that active anodal tDCS modified saccade error rates, and was associated with reduced selective attention to threat during 7.5% CO_2 induced anxiety; while the latency results, demonstrate that active vs. sham tDCS is associated with pro-saccade delayed threat engagement. rTMS data[328] supports the notion that left DLPFC stimulation is associated with reduced attentional engagement with angry faces – whereas stimulation of the right DLPFC has the opposite effect. There is also TMS evidence to suggest that TMS to the DLPFC was associated with longer pro-saccade and anti-saccade latency, and that this effect was more pronounced with pro than with anti-saccades[424].

The findings of our study align with those of a recent study which assessed the effect of a single session of DLPFC tDCS on a emotional processing, using the dot-probe task[418] - participants who received active tDCS with the same montage used in our study demonstrated reduced vigilance to threat compared to a sham tDCS group and to a group, that received active tDCS with the cathode over F8 (as opposed to F4 on the 10/20 system).

7.5.2. Self-report anxiety and affect

We predicted that:

- The 7.5% CO_2 challenge would be associated with significant increases in self-report anxiety and negative affect, and with reduced self report positive affect.

- Compared to sham tDCS, active DLPFC tDCS would be associated with reduced self-report anxiety.

The predicted 7.5% CO_2 challenge-related effects on subjective anxiety and affect were observed; and provide further support for using this challenge as a model for anxiety in healthy subjects. There were no significant effects of group on these self-report measures. The lack of effect of a single session of tDCS on self-report anxiety and affect may be due to the single-administration of tDCS in this study, and/or to the low-levels of anxiety in the cohort. This is in line with most published studies, which do not demonstrate acute tDCS effects on mood in healthy subjects[403,425,426]. For example, a placebo-controlled study of 17 healthy volunteers who received a 20-minute session of active vs. sham tDCS did not find any significant effects on mood[425]. A single 30 minute session of left DLPFC anodal tDCS was not associated with significant changes of subjective mood and anxiety as measured using VAS[426]. It was suggested that healthy subjects may have a 'ceiling effect' which prevents a single session of tDCS from having significant effects on mood, and requires a course of repeated treatments in order to produce measurable effects[427]. In contrast, chapter 2 of this thesis reviewed the evidence showing the effects of a course of tDCS on depression[253] and on anxiety in clinical populations. Evidence from studies of tDCS effects on cognition suggests that tDCS may require overnight consolidation in order to become effective[428].

In the current study, the recruited participants were healthy, with low anxiety levels, received a single session of tDCS, prior (rather than during) the anti-saccade task – all of these factors may have contributed to the lack of between-group differences found in this study.

7.5.3. Autonomic arousal

We predicted that the 7.5% CO_2 challenge would be associated with significant increases in autonomic arousal and self-report anxiety. These effects were observed, and provide further support to the use of this challenge as a model for anxiety in healthy subjects.

We predicted that a single session of active vs. sham tDCS would not be associated with significant differences in levels of autonomic arousal. There were indeed no significant between-group differences in heart-rate or blood pressure. This is in line with previously published studies which also did not find acute effects of tDCS on blood pressure and heart rate in healthy subjects[405,429].

A single session of active vs. sham tDCS was therefore associated with significant effects on the cognitive aspects of anxiety induced by the 7.5% CO_2 challenge, but not on subjective self-report anxiety or affect, nor on levels of autonomic arousal.

7.6. Limitations

There were several limitations to this study – (these are further discussed in section 8.5.3):

The tDCS studies utilise a single session intervention, which may not be sufficient to change anxiety levels – particularly in a cohort of well volunteers.

The tDCS montage used (anodal stimulation over the left dorsolateral prefrontal cortex), is commonly used in the treatment of depression, there is no widely accepted tDCS montage associated with reduced levels of anxiety.

8. CHAPTER 8 : GENERAL DISCUSSION

8.1. Review of thesis aims

This thesis describes a progression of steps, aimed at exploring the potential for using mindfulness and tDCS as treatment modalities for anxiety disorders (particularly for GAD).

8.2. Review of methods

The studies described in the thesis are linked by common methods:

8.2.1. Mindfulness

The mindfulness interventions used in chapters 3-5 were variations based on the core meditation skills of one-pointed concentration on the breath (FA), and choiceless awareness[430] (OM). Complex mindfulness interventions, including MBSR and MBCT, include these skills, alongside a range of other processes delivered as an intensive group package. The interventions used in this thesis were therefore a subset of mindfulness techniques, which were delivered less intensively. I first (Chapter 3) compared a course of FA vs. OM training vs. control, designed to enhance dispositional (trait) mindfulness, participants also receiving a guided meditation session prior to testing sessions, in order to create a state of mindfulness. Both FA and OM were effective in improving ANT executive control, but neither had a significant effect on self-reported anxiety. It was however unclear to what extent the OM group reverted to FA practice. I therefore opted to use an integrated FA+OM intervention (chapter 4) but there was no significant effect on attention to threat using the anti-saccade task, or on self-reported anxiety. This was potentially due to insufficient anxiety generated by the negative cues, but it is also possible that participants were mostly practicing FA during their home work sessions (as OM is a more advanced skill to practice), and were

therefore practicing FA at the testing session and being repeatedly distracted by the demands of the anti-saccade task. I subsequently (chapter 5) opted to test a single session of guided FA vs. OM – this is designed to create a mindfulness state (rather than improve trait mindfulness). Anxiety levels were increased using the 7.5% CO_2 inhalation model, and showed a clear hierarchy of anxiolytic effects (OM > FA > control). Considering all 3 chapters, it may be that:

1. A course of FA/OM/FA+OM training as delivered in chapters 3 and 4 is likely to enhance FA skills in novice meditators;

2. OM skills may be too complex to teach under these conditions;

3. The effect on dispositional mindfulness may not be sufficient to be measured using MAAS/KIMS;

4. The effects seen on executive function in chapter 3 and on self-reported anxiety in chapter 5 may be due to the effects of state-mindfulness induced by the in-session guided meditation, this may have been facilitated/enhanced by the training course in chapter 3, but was sufficient as a standalone intervention in chapter 5;

5. The mindfulness state induced by OM guided practice seems superior to that induced by FA guided practice in its acute anxiolytic effects.

8.2.2. tDCS

There are a number of methodological issues related to the use of tDCS in chapters 6 and 7: tDCS is an anatomically targeted intervention, exerting its effects on cortical structures underlying the electrodes. Montage is therefore an important factor, and in the absence of definitive evidence about the use of tDCS in anxiety, we employed the montage most often used for the treatment of depression – i.e. the left anodal, right cathodal DLPFC montage. It may be that this is not the optimal montage for

demonstrating effects on anxiety (indeed, the evidence in OCD, described in chapter 2, suggests that treatment of some anxiety disorders may require different tDCS montages). We attempted to blind both participants and operators as to whether each stimulation was active or sham – details of the blinding methods are described in section 6.3.3. Blinding was unsuccessful in both tDCS studies – blinding integrity data is described in sections 6.4.8 and 7.4.6. As for tDCS dose, we delivered a single session of tDCS using 2mA current for 20 minutes. The evidence supports the use of a course of repeated tDCS for clinical purposes, rather than tDCS given in a single session. Current and time settings used in depression studies have escalated over time – there is a gradual trend for higher current settings (up to 3mA) and longer sessions (30 minutes). Viewed in this light, the intervention we used can be compared to testing the effect of a single low-dose application of an antidepressant medication vs. placebo, on a cohort of healthy volunteers, with a view to considering whether this could be a treatment for anxiety disorders. Given this reservation, the significant effects on executive function and on attention to threat associated with active vs. sham tDCS are encouraging. The lack of effect on self-reported anxiety is consistent with the expectation that tDCS needs to be delivered as a course of repeated applications in order to exert clinical effects.

8.2.3. 7.5% CO_2 inhalation model

The 7.5% CO_2 inhalation model induces a state of anxiety. Chapter 5 demonstrated that in subjects inhaling 7.5% CO_2, a state of mindfulness induced by single session of guided meditation was associated with reduced self-reported anxiety, in the absence of significant effects on attention to threat and on measures of autonomic arousal. In chapter 7, a session of tDCS was associated with a different pattern: namely a

significant effect on attention to threat, in the absence of effects on self-reported anxiety and autonomic arousal. There may be a fundamental difference between the effects of a guided meditation – which induces a state of mindfulness even in the absence of a course of mindfulness training- and a session of tDCS, which affects underlying cognitive processing, but in the absence of a course of tDCS is not sufficient to reduce anxiety. There may also be a specific interaction between the 7.5% CO_2 inhalation procedures and the breathing focus employed by the FA technique. It is possible that focusing on the breath within an experiment involving a breath-related anxiogenic challenge could interfere with FA practice. It is therefore possible that the anxiolytic advantage of OM over FA may be accentuated in this particular experimental medicine model.

8.3. Review of main findings

8.3.1. Main findings - Literature review:

Chapters 1 contains an overview of the literature on anxiety, attention, and mindfulness. Anxiety disorders are an important cause of morbidity worldwide. Existing treatments for anxiety disorders have significant shortcomings and therefore, new treatments are needed. Anxiety impairs attentional control through effects on central executive functions. Attention is underpinned by the alerting, orienting, and executive attentional networks, whose activity can be measured using the ANT. The anti-saccade task is used for investigating performance efficiency and effectiveness of executive function. Mindfulness training has effects on executive function and on attention. Mindfulness-based interventions have a substantial evidence base for efficacy in

depression and a growing evidence base in anxiety disorders, with a good profile of acceptability and safety in both clinical and pre-clinical populations.

Chapter 2 describes the evidence for using tDCS in depression and in anxiety disorders: A meta-analysis of RCTs comparing active vs. sham tDCS in depression demonstrated that in patients with major depressive episodes, tDCS may offer an effective and tolerable alternative to antidepressant medication for those who do not wish to take medication, or cannot tolerate it. Current evidence does not support the use of tDCS in treatment resistant depression, or as an add-on augmentation treatment in addition to antidepressant medication or to Cognitive Control Training (CCT). There are no published RCTs of tDCS in anxiety disorders.

8.3.2.   Main findings - Chapters 3 – 5: Mindfulness studies

Chapter 3 – The effects of FA & OM mindfulness training on attention network function in healthy volunteers: This was the first study to examine the effects of FA vs. OM training on attention network performance. FA and OM training (but not the control group) were associated with significant improvements in the performance of the executive control attention network. There were no significant between group differences in orienting and alerting network performance. The predicted effects on orienting attention network performance in the presence of negatively-valenced cues were not demonstrated; this may have been due to negative word cues in the modified ANT not being disturbing enough to generate sufficient threat perception in this low-trait-anxiety cohort. There were no significant between group effects on self-reported anxiety, mindfulness, or attentional control.

Chapter 4 – Effects of integrated OM & FA mindfulness training on attention to threat:

Building on the finding of the previous chapter(that mindfulness training was

associated with improved executive function) this study utilised a strengthened

mindfulness intervention to gain a more detailed understanding of the effects of

mindfulness on attention to threat. The study did not demonstrate significant effects of

mindfulness training vs. control, on threat processing as measured using the anti-

saccade task. This may have been due to the combination of a low-trait-anxiety cohort,

with a task that did not place sufficient cognitive demands on participants in non-

anxiety provoking conditions. There were also no significant effects on self-report

measures of anxiety, attention-control, worry, or mindfulness.

Chapter 5 – The effects of a single session of mindfulness meditation on attention

control in the 7.5% CO_2 inhalation challenge - a novel experimental human model of

anxiety: In order to gain a better understanding of the effects of mindfulness on

anxiety and on attention to threat, this study employed the 7.5% CO_2 challenge so as to

increase the level of anxiety during the anti-saccade task. The mindfulness session was

delivered within the testing session to induce a *mindfulness state* (in contrast to a

course of mindfulness training aimed at setting up a *mindfulness-trait*). The study did

not demonstrate significant between-group differences in attention control as

measured on the anti-saccade task during 7.5% CO_2 challenge. Saccade accuracy rates

across both groups were significantly lower on anti-saccade trials with neutral vs.

negative cues - this may be related to increased threat-attention related to negative

(but not to neutral) stimuli. Importantly, this was the first study to demonstrate that

components of contemporary mindfulness interventions can reduce anxiety in an

experimental human healthy subject model of anxiety. A single 10-minute intervention

with either FA or OM meditation training was associated with significant self-reported anxiolytic effects in an experimental 7.5% CO_2 model of anxiety in healthy human subjects. OM was associated with more marked anxiolytic effects than FA. There were no significant between-group differences in measures of autonomic arousal.

8.3.3.   Main findings - Chapters 6 – 7: tDCS studies

Chapter 6 – The effect of a single session of active vs. sham tDCS on Attention Network function: The design of this study parallels that of the study in chapter 3, substituting tDCS for a mindfulness intervention. The study demonstrated a significant superiority of executive control (but not orienting or alerting) network performance following active vs. sham tDCS. This finding mirrors the finding in chapter 3, in which mindfulness training (FA & OM) was associated with a similar pattern of effects on attentional network function. There were no significant between-group differences in self-reported affect and anxiety, or in measures of autonomic arousal. The lack of effect on these self-report measures accords with the evidence base, which suggests that in order to induce changes in mood, a course of repeated tDCS is necessary, and a single session is unlikely to be associated with significant changes, there is also evidence that tDCS is not associated with mood change in healthy subjects (see discussion in section 7.5.2).

Chapter 7 – the effects of a single session of active vs. sham tDCS on attention control in the 7.5% CO_2 challenge: This study employed the same tDCS intervention used in Chapter 6, and the same experimental design as in chapter 5, using the 7.5% CO_2 challenge to model anxiety, while measuring attention control using the modified anti-saccade task. Following active (but not sham) tDCS, the error rates in negatively-cued

(pro- and anti-saccade) trials were lower than in neutrally-cued trials (p = 0.04); this may reflect reduced attention to threat following active tDCS. There were no significant between-group differences in self-reported affect or anxiety, and no significant between group differences in measure of autonomic arousal.

	Intervention	Mindfulness	Subjective anxiety	Autonomic arousal	ANT	Anti-saccade task
Chapter 3	Meditation training course FA vs. OM vs. control vs. relaxation control	No significant between-group differences	No significant between-group differences	Not measured	• Executive function significantly improved in FA & OM meditation training but not in control group. • No difference in orienting and alerting network function.	Not measured
Chapter 4	Integrated FA & OM meditation course training vs. test-retest control	No significant between-group differences	No significant between-group differences	Not measured	Not measured	No significant between-group differences
Chapter 5	A single in-session guided meditation (OA vs. FM vs. relaxation control) 7.5% CO_2 Challenge	No significant between-group differences	• CO_2 induced Increase in anxiety: OM<FA<Control • Reported anxiety levels post CO_2: Control > OM (trend towards Control > FA)	No significant between-group differences	Not measured	No significant between-group differences
Chapter 6	A single session of active vs. sham anodal left DLPFC tDCS	Not measured	No significant between-group differences.	No significant between-group differences.	• Executive function: Active tDCS superior to sham tDCS. • No difference in orienting and alerting network function.	Not measured
Chapter 7	A single session of active vs. sham anodal left DLPFC tDCS. 7.5% CO_2 Challenge	Not measured	No significant between-group differences.	No significant between-group differences.	Not measured	• Active (but not sham) tDCS was associated with significantly lower error-rates in negatively-cued (pro and anti-saccade) compared to neutrally-cued trials. • Trend towards active tDCS to be associated with higher accuracy in threat-cued anti-saccades vs. neutrally-cued anti-saccades • Trend for longer pro-saccade latencies towards negative cues in the active vs. sham tDCS

Table 8.1: Summary of key findings across all studies reported in this thesis. FA= Focussed attention meditation; OM = Open Monitoring meditation

8.4. Contributions to scientific understanding

8.4.1. Added knowledge

8.4.1.1. Added knowledge– Literature review

The meta-analysis of active vs. sham tDCS in depression provided an optimised summary of the evidence. In comparison to previous meta-analyses, it included 43% more RCTs, and increased the number of participants by 23%. This enabled increased power to detect small effects, and better precision in terms of pooled confidence interval width (21% improvement). The meta-analysis significantly strengthened the evidence for a clear superiority of active tDCS over sham tDCS in the treatment of MDE. In order to offer a consistent approach, we analysed all outcomes at the point of intensive tDCS treatment cessation which had not been done previously. We were able to assess the effect of adding tDCS to CCT, this was not previously meta-analytically addressed. The meta-analysis also includes power and precision analyses, which highlight the sub-optimal power of most RCTs included, and offers guidance regarding future trial design (see section 8.6). The meta-analysis of active vs. sham tDCS in depression provided several new insights. The current body of evidence does not support the use of tDCS in treatment resistant depression, nor does it support the use of tDCS as an add-on augmentation treatment for depressed patient who are already taking an antidepressant or undergoing Cognitive Control Training (CCT) – this is the first meta-analytic assessment of tDCS as augmentation for a non-pharmacological treatment in depression.

8.4.1.2.   Added knowledge – Mindfulness studies

The mindfulness intervention studies (chapters 3-5) contributed new findings by demonstrating for the first time that FA and OM mindfulness training improves executive control attention network function as measured on the ANT (chapter 3), that OM and FA mindfulness training have an anxiolytic effect when combined with the 7.5% CO_2 model of human anxiety (chapter 5), and that the anxiolytic effects of OM are stronger that those of FA training (chapter 5). Two important findings are that a single session of guided mindfulness training has a significant self-reported anxiolytic effect, and that OM out-performs FA in its efficacy for anxiety reduction during 7.5% CO_2 inhalation.

8.4.1.3.   Added knowledge – tDCS studies

Chapter 6 is the first study to demonstrate that a single session of tDCS is associated with enhanced function of the executive control (but not orienting or alerting) attentional network, without effects on self reported anxiety, attentional control or autonomic arousal. This is a similar selective pattern to that observed following a course of mindfulness training, described in chapter 3. Chapter 7 demonstrated that active vs. sham tDCS modified saccade error rates (significant accuracy increase in threat-cued saccade trials, and trend towards higher accuracy in threat-cued vs. neutrally-cued anti-saccades), and was associated with reduced selective attention to threat during 7.5% CO_2 induced anxiety; saccade latency results demonstrated that active vs. sham tDCS.

The findings that a single session of tDCS is associated with executive control network function, and that in the context of the 7.5% CO_2 inhalation model, with reduced attention to threat, both imply that tDCS may have potential as a treatment for anxiety disorders. In particular the effects may be targeting mechanisms underlying state-anxiety. The evidence in depression is that the effectiveness of tDCS follows a 'dose-response' curve, and that a therapeutic effect requires a course of repeated tDCS. This may indicate that a course of tDCS is needed for the treatment of anxiety disorders.

8.5. Limitations and areas of current uncertainty

8.5.1. Limitations – Literature review

The main limitation of the meta-analysis of tDCS in depression is the low number of participants in most of the included trials. As demonstrated by our precision and power calculations, all but one of these trials [275] are probably underpowered. This is likely to explain the lack of separation between active and sham tDCS in terms of categorical response and remission outcomes; as well as limiting the number of moderators reaching statistical significance. The meta-analysis was based on outcomes at the end of active tDCS treatment – this was not always the primary outcome measure of the original trials, and may have contributed to under estimating the effects of tDCS in the meta-analysis. There is a lack of evidence regarding longer-term outcomes of tDCS in the acute and maintenance treatment of depression. The lack of published RCTs is a major limitation in the evidence base for using tDCS in anxiety disorders.

8.5.2. Limitations – Mindfulness studies

Study design: mixed designs with baseline testing to provide reference for any within group change as well as between group variation. Power calculations are described within each chapter (see sections 3.3.1, 4.3.1, and 5.2.1 respectively).

It appears likely that the negatively valenced cues used in the ANT (chapter 3) and in the anti-saccade task (chapters 4 and 5) were not distressing enough to elicit a sufficient anxiety response in the low trait-anxiety cohorts we recruited. Chapter 3 did not therefore demonstrate the predicted effects of mindfulness training on orienting attention network performance in the presence of negatively-valenced cues; and chapter 4 did not demonstrate significant differences between the mindfulness and control groups in terms of anti-saccade task performance, and self-report measures of state anxiety, worry and attention control. Another key limitation is the repeated lack of significant effect of mindfulness training on self-report measures of dispositional mindfulness in all 3 mindfulness studies. This could be related to inherent problems with measuring mindfulness by self-report (as discussed in chapter 1), to the specific mindfulness self-report measures that we used (MAAS and KIMS), or to insufficiently robust mindfulness interventions. The limitations of each mindfulness study are discussed within the relevant chapters (see sections 3.8, 4.6, and 5.5 respectively).

Study design: mixed designs with baseline testing to provide reference for any within group change as well as between group variation.

Participants were a young, healthy, predominantly female sample, naïve to interventions and consequently might find it challenging to engage with some of the interventions that were delivered. There is also a risk that they would perform at ceiling on behavioural performance measures including reaction time on the attention network test and error

rates on the antisaccade task. Effects may have been supressed by responder bias on subjective measures of mood and anxiety.

8.5.3. Limitations – tDCS studies

tDCS stimulation was delivered using a DLPFC montage – due to the anatomical structures underlying attentional networks, this montage is likely to be preferentially selective to enhancing the executive control network, rather than the orienting or alerting networks. Deficits in executive control network function are associated with state-anxiety (in contrast to trait-anxiety, which is associated with orienting network impairments)[74]: therefore trait-anxiety effects that could potentially be achieved with other tDCS montages, may have been missed. The tDCS montage used (anodal stimulation over the left dorsolateral prefrontal cortex), is commonly used in the treatment of depression, there is no widely accepted tDCS montage associated with reduced levels of anxiety.

The tDCS studies were limited to a single session of tDCS and the likelihood is that a single session would be ineffective in reducing anxiety, and only serve to modulate subtle cognitive mechanisms underlying anxiety, rather than alter overall mental state. Participants were a young, healthy, predominantly female sample. There is a risk that they would perform at ceiling on behavioural performance measures including reaction time on the attention network test and error rates on the antisaccade task. Effects may have been supressed by responder bias on subjective measures of mood and anxiety.

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8.6. Implications for future research

8.6.1. Implications for academic research

8.6.1.1. Implications for academic research - Literature review:

The power and precision analyses in the meta-analysis of tDCS for depression indicated that for an individual study to detect the summary effect at the p =.05 level at 80% power, an N of at least 173 would be required in both the treatment and control group. Future studies may wish to focus on enhancing the precision of their interval-based estimates within practical and financial constraints, rather than placing too much stock in conclusions based on null hypothesis significance testing. In this context, *a priori* power and precision analyses (and explicit reporting of these values) are recommended to enhance interpretation of data relating to tDCS efficacy in future RCTs.

8.6.1.2. Implications for academic research - Mindfulness studies

The finding in Chapter 3 that FA and OM mindfulness training enhanced executive control attentional network performance, when considered in the light of the important role of attentional control deficits in anxiety-related cognitive processing difficulties, lends further support to future studies aimed at clarifying the relationship between specific mindfulness skills (FA, OM, or integrated FA+OM) and executive network function. The findings in chapter 5 of the anxiolytic superiority of OM over FA, and the specific difficulties of using FA while attending to cognitive tasks, suggest that integrated FA+OM training (as delivered in chapter 4) may lend itself better for use with a wider range of outcome measures, while maximising anxiolytic potential. Chapter 5 demonstrated that while reported anxiety levels decreased in the FA and OM

groups, there was no significant difference in measures of autonomic arousal – this uncoupling is consistent with the stated ethos of mindfulness as seeking to accept rather than to modify potentially aversive experiences, and suggests that future studies may wish to emphasise self-reported anxiety rather than autonomic arousal measures as outcome measures in studies of mindfulness and anxiety. Future research may also consider whether there are particular advantages in targeting mindfulness interventions selectively at individuals who demonstrate particular anxiety-related deficits in executive control. For example, would a particular pattern of executive control dysfunction serve as a marker for mindfulness-related recovery? It might also be helpful to study whether early signs of improvement in executive control function might serve as predictors for subsequent symptomatic improvement in anxiety disorders.

8.6.1.3.   Implications for academic research - tDCS studies

The therapeutic effects of tDCS in depression suggest a dose-response curve in terms of intra-session factors (e.g. current setting, session duration) and factors external to the session (including inter-session interval, number of sessions, tDCS montage etc.). Modifications of some of these factors may enhance the magnitude and stability over time of the enhancement in executive function, as well as lead to enhanced effects on reported anxiety. There is also potential for research exploring the potential for improving executive functions in healthy individuals, including cognitive enhancement in the context of academic and occupational domains.

8.6.2.   Implications for clinical research

8.6.2.1. Implications for clinical research - Literature review:

The meta-analysis of tDCS in depression suggests that future clinical research may be better targeted at the earlier stages of depression treatment pathways. The lack of evidence for efficacy of tDCS in TRD or as augmentation for those already taking antidepressant medication, or undergoing CCT, combined with robust evidence for efficacy in non-treatment resistant depression, and considered alongside its good acceptability and tolerability all point towards potential advantages for exploring the use of tDCS as monotherapy in early stage depression – perhaps even in primary care settings (potentially offering treatment in participants' homes). It may also be useful to explore tDCS for relapse prevention following a course of ECT in depression.

8.6.2.2. Implications for clinical research - Mindfulness studies:

The finding in Chapter 3 that FA & OM mindfulness training enhanced executive control attentional network performance, when combined with the significant anxiolytic effects of mindfulness training as demonstrated in chapter 5 lends further support to the therapeutic potential of brief mindfulness-based interventions in anxiety. This is further strengthened by the advantages of mindfulness-based interventions in terms of their acceptability, growing evidence base for efficacy and effectiveness, and the potential for delivering them in a variety of contexts including group-settings, and home-based training. The finding in chapter 5, that in terms of anxiolytic effect, OM was superior to FA, would support emphasising the OM component (either on its own, or integrated with FA) in future research into mindfulness interventions for anxiety disorders, developing therapeutic interventions that aim to improve acceptance and

emotional openness towards anxiety symptoms, rather than focusing on distraction strategies.

8.6.2.3. Implications for clinical research - tDCS studies

There are early indications that tDCS might provide an additional treatment option for anxiety disorders. Considering the need for new treatment modalities, and the highly acceptable nature of tDCS, it is important to undertake RCTs of tDCS in anxiety disorders. Changing and/or combining tDCS montages may enable targeting of orienting as well as executive control networks. This could enhance the effect on anxiety by combining effects on state and trait anxiety. The optimal set of tDCS parameters for treating anxiety disorders would need to be explored (and may vary across different anxiety disorders). Furthermore, a range of other disorders is associated with executive function impairments (including schizophrenia, attention deficit and hyperactivity disorder (ADHD), frontal and Alzheimer's dementia, and learning disability) and there may be therapeutic applications for tDCS in these disorders.

8.7. Implications for clinical practice

8.7.1. Implications for clinical practice – Literature review

The meta-analysis of tDCS in depression supports the use of tDCS for the treatment of MDE in the context of unipolar and bipolar mood disorders. The evidence points towards using tDCS as a monotherapy in the early stages of depression treatment pathways, rather than for augmentation in treatment resistant depression. There may

be an advantage for offering tDCS as a first-line treatment for depression in primary

care settings. In this context the treatment may be delivered in patients' homes. There

is insufficient evidence to make recommendations regarding the use of tDCS for the

treatment of anxiety disorders. There is sufficient evidence to support the use of

mindfulness-based interventions (particularly MBSR and MBCT) in the treatment of

depression (see discussion in section 1.4.3). There is also evidence to support the use

of mindfulness interventions (particularly MBSR and MBCT) in the treatment of anxiety

disorders (see discussion in section 1.4.4).

8.7.2.   Implications for clinical practice – Mindfulness studies

The finding in Chapter 3 that FA and OM mindfulness training enhanced executive

control attentional network performance, when combined with the significant

anxiolytic effects of mindfulness training as demonstrated in chapter 5, and with the

finding in chapter 4 that an integrated FA and OM mindfulness training was practical

and acceptable, suggest that emphasizing OM components when delivering current

mindfulness interventions for anxiety disorders may be more effective for producing

anxiolytic effects. Those developing future mindfulness-based approaches for anxiety,

may wish to enhance OM or OM&FA integrated components, in light of these findings.

Future treatments may include online / mobile application-based mindfulness

interventions, as this thesis suggests that a web-based intervention was effective,

practical, and well tolerated.

8.7.3.   Implications for clinical practice – tDCS studies

The evidence base supporting the use of tDCS in the treatment of anxiety disorders is not sufficient to enable firm conclusions to be drawn. In the future, tDCS may represent a treatment option for anxiety disorders, either as mono-therapy, or in combination with pharmacological and/or non-pharmacological treatment modalities.

8.8. Next steps

The studies described within this thesis identified two main clinical issues that require further investigation:

First, the use of tDCS as a monotherapy in early stages of depression treatment pathways: our group is involved in a multi-centre grant application for a study to explore this area by offering primary care patients with depression, treatment with tDCS in their own homes. Second, the need for new treatment modalities for anxiety disorders (including obsessive-compulsive disorder). Case reports and pre-clinical studies suggest that tDCS may offer a tolerable and acceptable alternative to existing treatments, but there have not yet been any published RCTs of tDCS in anxiety disorders: our group is currently involved in a multi-centre application for a study to explore the use of tDCS in OCD.

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