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UNIVERSITY OF SOUTHAMPTON

FACULTY OF SOCIAL, HUMAN AND MATHEMATICAL SCIENCES

PSYCHOLOGY

**Targeting neuropsychological mechanisms in anxiety - an evaluation of transcranial
direct current stimulation and attention training in the 7.5% carbon dioxide
experimental model of anxiety.**

by

Joanna Astrid Miler

Thesis for the degree of Doctor of Philosophy

September 2015

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ABSTRACT

FACULTY OF SOCIAL, HUMAN AND MATHEMATICAL SCIENCES

Psychology

Thesis for the degree of Doctor of Philosophy

TARGETING NEUROPSYCHOLOGICAL MECHANISMS IN ANXIETY - AN EVALUATION OF TRANSCRANIAL DIRECT CURRENT STIMULATION AND ATTENTION TRAINING IN THE 7.5% CARBON DIOXIDE EXPERIMENTAL MODEL OF ANXIETY.

Joanna Astrid Miler

Neuropsychological models of anxiety suggest that deficits in prefrontal mechanisms underlie maladaptive biases in attention control and hypervigilance to threat. Current first line treatments do not target those mechanisms (e.g. Bar-Haim, 2010). Transcranial direct current stimulation (tDCS) - a non-invasive brain stimulation modality which alters cortical tissue excitability - and Attentional Bias Modification Training (AMBT) - a novel computer-based attention training protocol which implicitly modifies biased attentional patterns - both offer a way to target those mechanisms, and may represent alternative treatment options for patients with mood and anxiety disorders. Numerous studies suggest beneficial effects of tDCS in the treatment of depression (e.g. Brunoni et al, 2012; Fregni et al, 2006; Fregni et al, 2006b; Boggio et al, 2008a) but despite high comorbidity and shared neurocognitive mechanisms between depression and anxiety (e.g. Hirschfeld et al, 2001; Lamers et al, 2011) no studies to date have evaluated potential beneficial effects of tDCS in anxiety.

Experimental models of anxiety (for example the 7.5% CO₂ model), which temporarily mimic anxiety symptoms in healthy samples, can provide a useful way of investigating the effectiveness of such novel treatments. This thesis presents a series of experiments that use the 7.5% CO₂ model to evaluate the therapeutic potential of treatments that target attentional mechanisms. Study One (Chapter 2) investigates the effects of tDCS on attention network function in healthy (unchallenged) humans and provides evidence that 20 minutes of left anodal tDCS of the DLPFC results in superior executive function as compared to sham stimulation, in the absence of mood changes. Study Two (Chapter 3) investigates the effects of tDCS on the response to the CO₂ challenge and attention control in an emotional antisaccade task. Results suggest that tDCS administered immediately prior to 20 mins of a 7.5% CO₂ inhalation reduces erroneous eye-movements towards threat images relative to neutral images relative to sham. Study Three (Chapter 4) similarly investigates the effects of ABMT on the response to the CO₂ challenge and antisaccade performance: contrary to predictions and previous evidence, the ABMT protocol did not train an attentional bias, did not alter response to CO₂ challenge, nor affect antisaccade performance.

The goal of the thesis extended beyond evaluation of potential treatments for anxiety, and for the first time, in a within-participants design examined changes in cortical brain activation during the 7.5% CO₂ challenge vs. inhalation of air (Study Four, Chapter 5). A different activation pattern between the CO₂ and air inhalations was observed, characterised by significantly lower alpha activity in parietal and occipital regions, paired with significantly higher gamma and theta activity across all sites, suggesting that the CO₂ model induces brain activation changes broadly consistent with those observed in clinical anxiety. The thesis ends with a general discussion, and considers implications of the findings for future research.

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DECLARATION OF AUTHORSHIP

I, *Joanna Astrid Miler*, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The Evaluation of Transcranial Direct Current Stimulation (tDCS) and Attentional Bias Modification Training as Novel Treatments for Generalised Anxiety Disorder - a Further Validation of the 7.5% Carbon Dioxide Model.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission:

Signed:

Date:.....

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Definitions and Abbreviations

ANT - Attentional Network Test	RT – reaction time
APA – American Psychiatric Association	CBT – Cognitive Behavioural Therapy
ASI – Anxiety Sensitivity Index	ACS - Attentional Control Scale
BMI – Body Mass Index	SSAI - Spielberger State-Anxiety Inventory
CO₂ - carbon dioxide	DLPFC – dorsolateral prefrontal cortex
DBP – Diastolic Blood Pressure	FEF – Frontal Eye Fields
dmPFC - dorsomedial prefrontal cortex	NICE - National Institute for Health and Care Excellence
DSM – Diagnostic & Statistical Manual of Mental Disorders	GAD – Generalised Anxiety Disorder
EEG - Electroencephalography	Hz - Hertz
EOG - Electrooculography	O₂ - oxygen
ER – error rate	ERP - event-related potential
fMRI – Functional magnetic resonance imaging	HPA – hypothalamic–pituitary–adrenal axis
GAD7 – Generalised Anxiety Disorder - 7	PANAS - Positive and Negative Affect Schedule
GAFs – Global Assessment Functioning Scale	5-HT – 5-hydroxytryptamine
HA - Alternative Hypothesis	MINI - Mini-International Neuropsychiatric Interview
HDRS - Hamilton Rating Scale for Depression	BDI - Beck Depression Inventory
HR – Heart Rate	SBP – Systolic Blood Pressure
LPFC – Lateral prefrontal cortex	MDD – major Depressive Disorder
LPP - late positive potential	ACC – anterior cingulate cortex
LTP – Long Term Potentiation	LTD – Long Term Depression
MEP - Motor evoked potential	PIM - person-identity-matching (task)
mPFC - medial prefrontal cortex	OCD – Obsessive Compulsive Disorder
N₂ - nitrogen gas	FFT - fast Fourier transform
NAT – Negative Automatic Thoughts	ADM – Affective Decision Mechanism
NMDA - N-Methyl-D-aspartic acid	ctDCS - Cerebellar Transcranial Direct Current Stimulation
OAS - off-target active control condition	SAD – Seasonal affective disorder
PET - positron emission tomography	rCBF - regional cerebral blood flow
Pre-SMA – pre-supplementary motor area	POMS – Profile of Mood States
PTSD – Post-Traumatic Stress Disorder	STAI-T – Spielberger Trait-Anxiety Inventory

RAM – Resource Allocation Mechanism

rTMS - Repetitive Transcranial Magnetic Stimulation

SSRI – Selective serotonin re-uptake inhibitor

tDCS – transcranial direct current stimulation

TMS – Transcranial Magnetic Stimulation

VAS – Visual Analog Scale

VLPFC - Ventrolateral prefrontal cortex

vmPFC - ventromedial prefrontal cortex

Vs. – versus

CCT - Cognitive Control Therapy

ACT – Attentional Control Theory

SD – Standard Deviation

PFC – prefrontal cortex

ABMT – Attentional Bias Modification Training

ADM – abductor minimi digiti

PSWQ - Penn State Worry Questionnaire

BOLD - Blood-oxygen-level dependent

mA - milliamp

µg - microgram

i. Chapter 1

Literature Review.

1.1 Anxiety

Anxiety has been conceptualized in many ways. Lewis (1970) defined anxiety “*as an emotional state, with the subjectively experienced quality of fear as a closely related emotion*” (p. 77) and conceptualised it as an unpleasant, negative emotion, which is: out of proportion to the threat, future directed, and involving both subjective aspects and manifesting bodily disturbances. Throughout the literature anxiety has been defined as a trait, a state, a stimulus, a response, a drive, and a motive (Endler & Kocovsky, 2001).

Individuals vary in the degree to which they experience anxious responses (a tendency stable over time) – a trait closely related to the personality dimension of neuroticism (e.g. Eysenck & Eysenck, 1985; Watson & Clark, 1984). The level of anxiety felt by an individual *at that moment* is distinct from trait anxiety. Thus anxiety can be subdivided into two types: state (fear, discomfort, and corresponding autonomic arousal; a temporary experience; a feeling at the time of a perceived threat) and trait (feelings of stress, worry, discomfort experienced on a day to day basis; a constant experience) (Spielberger & Sydeman, 1994). This suggestion for a state-trait anxiety distinction was originated in 1966 by Spielberger, with trait anxiety defined as a general propensity to be anxious or respond anxiously, and state anxiety as a transitory emotion characterized by physiological arousal and consciously perceived feelings of apprehension, dread, and tension. Trait anxiety was suggested to not be directly observable, but rather expressed as state anxiety during stress (Reiss, 1997). The trait–state distinction has received recognition in the psychological literature in explaining the tendency for individuals high in trait anxiety to experience more state anxiety than those low in trait anxiety, over many decades (e.g. Dreger, 1985, Endler, 1983, Endler, 1997 and Spielberger, 1985).

Individuals with elevated trait anxiety experience increases in state anxiety in response to even moderately threatening stimuli (e.g. Koster et al, 2005) and show the same pattern of responses as anxiety disorder patients in many cases - for example both state and trait anxiety have traditionally been characterised by deficits in cognitive control (e.g. Lazarus, 1991; Mandler, 1984) and hypervigilance to threat (e.g. Eysenck, 1997; Williams et al, 1997).

However, it is important to note that elevated trait anxiety is in itself not an anxiety disorder, nor does it necessarily lead to one. In healthy individuals fear and anxiety can in fact facilitate action

in an effort to maintain well-being and to preserve life (e.g. Sylvers et al, 2011; Lang, Davis & Öhman, 2000). Individual differences in trait anxiety are considered along a continuum, where too low levels of trait anxiety may be maladaptive (may fail to orient to/react with state anxiety to an actual threat), medium levels being optimal as state anxiety would be appropriately triggered in response to genuine threat, but where high trait anxiety becomes problematic, leading to frequent increases in state anxiety (in the absence of threat), recurrent/intrusive worry and avoidance behaviours. In extreme cases anxiety symptoms may produce levels of functional impairment that warrant clinical assessment and intervention.

An anxiety disorder is an umbrella term for multiple forms of abnormal or inappropriate anxiety reaching a clinical level. Each type of anxiety disorder has its own characteristics and symptoms but overlapping features exist and comorbidity between anxiety disorders is common (e.g. Wittchen et al, 1994; Noyes, 2001). DSM-5 recognises ten types of anxiety disorders: Adjustment Disorder, Specific Phobia, Social Anxiety Disorder (SAD), Panic Disorder, Agoraphobia, Separation Anxiety Disorder, Substance-Induced Anxiety Disorder, Anxiety Disorder Attributable to Another Medical Condition, Anxiety Disorder Not Elsewhere Classified and Generalized Anxiety Disorder (GAD) (APA, 2013).

GAD is an interesting disorder as there is no one specific trigger or cause for the experienced worry and anxiety (in contrast to for example social phobia where the worry concerns social interactions). GAD's reported prevalence rates are approximately 1.6% and 5.8% for current and lifetime prevalence respectively (e.g. Wittchen, Zhao, Kessler & Eaton, 1994; Khan & Macalusco, 2009). Early research suggested that females outnumber males by a 5:2 ratio (Blazer, George & Hughes, 1991), but more recent studies suggest that men are "catching up", with lifetime and 12-month male:female prevalence ratios for GAD at 1:1.9 and 1:2.2, respectively (e.g. Vesga-Lopez et al, 2008). For a diagnosis of GAD symptoms must be unrelated to any other medical or mental health conditions or substance use. The hallmark of GAD is the "presence of excessive anxiety and worry about a variety of topics, events, or activities. Worry occurs more often than not for at least 6 months, and is clearly excessive" (DSM-5, APA, 2013). Excessive worry means worrying even when there is nothing wrong, or worrying disproportionately to any actual risk. This typically involves spending a high percentage of waking hours worrying, which may be accompanied by reassurance-seeking from others (APA, 2013). Many individuals with GAD also experience physiological symptoms such as sweating, nausea or diarrhoea. As anxiety and its associated symptoms are hard to control, it typically impairs performance of day-to-day activities and responsibilities and subsequently lead to problems in important areas of life (APA, 2013). In GAD the worry is accompanied by at least 3 of the following physical or cognitive symptoms:

- a) Edginess or restlessness.
- b) Being easily tired and more fatigued than usual.
- c) Impaired concentration or feeling of mind going blank.
- d) Irritability.
- e) Increased muscle aches or soreness.
- f) Difficulty sleeping.

The features of GAD include cognitive biases, such as an 'attentional bias' to negative and threatening stimuli, with anxious individuals displaying a hypervigilance to threat (e.g. Mogg, Bradley & Williams, 1995). This attentional bias may contribute to the uncontrollable worry and a ruminative thinking style (continuous elaborated processing of negative information) in anxious individuals with rumination defined as "behaviours or thoughts that focus an individual's attention on his or her negative mood, and [on] the possible causes and consequences of that mood" (Nolen-Hoeksema, 2000). Thus attention (and biases thereof) may play a significant role in aetiology and maintenance of maladaptive thinking styles and pathological worry that characterise GAD.

Cognitive Models of Anxiety

A number of cognitive models of anxiety suggest that biases in information processing have a crucial role in the aetiology and maintenance of disorders such as GAD (e.g. Beck, 1976; Eysenck, 1992). All models highlight the role of cognitive biases in anxiety, and in particular biases in selective attention and attention control.

1.1.1 Beck's Schema Theory

The Schema Theory of emotional disorders developed by Beck (1976) and Beck et al (1985) contributed to the development of Cognitive Behavioural Therapy (CBT) - a first line treatment for GAD and depression (e.g. Butler et al, 1991). The model consists of three core elements: schemas (core beliefs serving as filters during interpretations of events), negative automatic thoughts (NATs; intrusive thoughts or images) and systematic cognitive biases. Anxious individuals were suggested to hold a schema of the world as a dangerous place, resulting in inaccurate perceptions of danger, and resulting in activation, control and modulation of emotional behaviour (e.g. Beck, 1976; Beck et al, 1979, 1986). Activation of the schema was proposed to become more likely during times of increased anxiety/stress, and lead to danger-relating NATs. Anxious individuals

were also proposed to exhibit cognitive biases in all aspects of information processing: (increased attention to danger cues – attentional bias; likelihood to interpret neutral stimuli as threatening – interpretational bias; and recall danger related memories – recall bias), congruent with the danger schemas.

Beck's Schema Theory has made a valuable contribution as evidence suggests that anxious individuals exhibit schema-congruent processing that influences behaviour and cognition (see a review by Eysenck et al, 1992). However the model has a number of limitations. For example no direct or independent evidence of the existence of a "schema" exists (e.g. Eysenck & Keane, 2013).

1.1.2 Bower's Associative Network Theory

Bower's (1981) theory concerns the relationship between mood and memory, later extended by Bower and Cohen (1982), Gilligan and Bower (1984) and Bower (1987). The key notion is the idea that long-term memory is a semantic associative network, where various concepts (both memories and emotions) are represented as "nodes". When any node is activated by internal or external stimuli, the activation selectively spreads to other related nodes (e.g. "sad" emotion node may be connected to sad memory, "happy" node to a happy memory) (e.g. Eysenck & Keane, 2013). According to the model, the activation of the emotion node would increase the accessibility of associated nodes, producing a bias favouring the information congruent with the emotional state, and an increase in anxious mood would result in increased activation of danger related material in memory. Moreover, the theory predicts mood congruent learning is characterised by selective encoding, retention and retrieval of negative material.

Bower's associative network theory and its recent revisions (1982; 1987) also has limitations, most notably over-simplicity in its treatment of memories and emotions as theoretically similar (categorising them both as nodes on the semantic network) (Eysenck & Keane, 2013). Moreover evidence suggests that by recalling mood incongruent material, those experiencing negative mood seem to actually improve their moods (Erber & Erber, 1994; Rusting & DeHart, 2000). Other evidence suggests that anxiety is characterised by biases in attention, but not memory (e.g. Mogg et al, 1987; MacLeod, 1990), and that memory bias is present in some, but not across all anxiety disorders (e.g. Cloitre et al, 1995; Coles & Heimberg, 2002).

1.1.3 Williams, Watts, MacLeod, and Mathews Model of Attentional Bias (1988)

Williams et al (1988) for the first time made a distinction between anxiety and affective disorders in terms of the presence of cognitive biases. They suggested that specific cognitive biases are

attributed to different emotional disorders - with anxiety characterised by a bias favouring threat stimuli in pre-attentive processes and in selective attention, and depression by a post-attentive memory bias.

Williams et al (1988) proposed two mechanisms (see Figure 1): the Affective Decision Mechanism (ADM), believed to assess threat value of environmental stimuli; and the Resource Allocation Mechanism (RAM), proposed to determine the allocation of processing resources. The ADM output was thought to be affected by current mood state, increasing the stimulus' threat value and then subsequently fed into RAM. The model suggested trait anxiety to influence the direction of the attentional biases to threat, when the output of ADM increases either due to increased state anxiety or stimulus threat value, high and low anxious individuals were predicted to differ in their attentional bias - high anxious individuals by orienting towards and low anxious away from threat (Williams et al, 1988). These respective biases were thought to increase with state anxiety (consistent with a state x trait interaction).

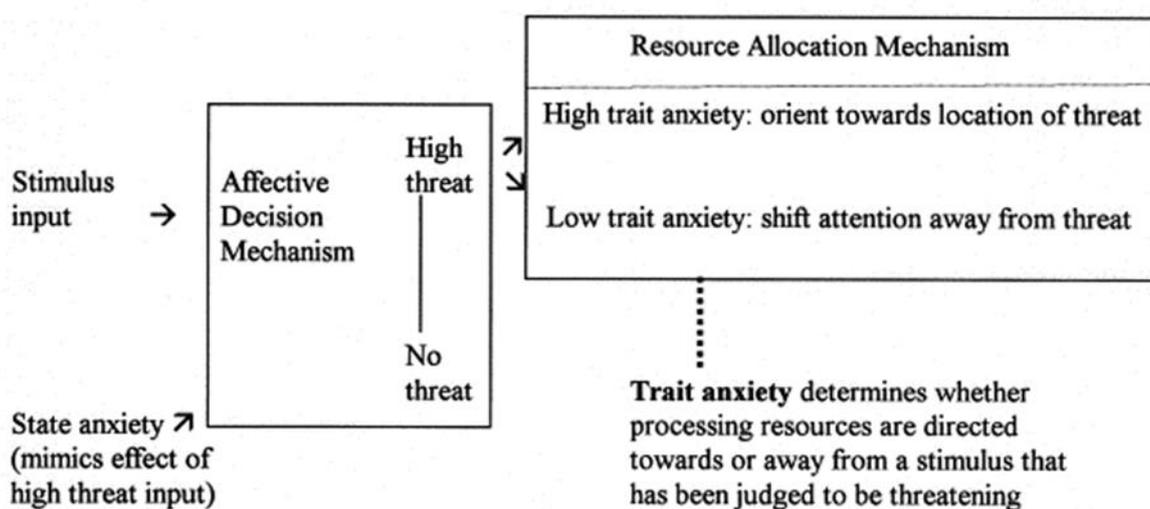


Figure 1 Williams, Watts, MacLeod, and Mathews Model of Attentional Bias (1988).

The original version of this model failed to explain the critical role of competition in attention. Anxious individuals identify threatening stimuli faster than neutral stimuli only when stimuli are presented in pairs (e.g. MacLeod & Mathews, 1991; Mogg, Mathews, Eysenck, & May, 1991), which implies that anxiety is characterised by the attentional priority assigned to threat over other cues, rather than by the efficiency of processing threatening information (Mathews & Mackintosh, 1998).

To address this problem, Williams et al. (1996; see also Williams, Watts, MacLeod, & Mathews, 1997) proposed that in the ADM emotional "tags" strengthen the activation of units representing threat (obtained either from biological preparedness or from prior learning) (Mathews & Mackintosh, 1998). The "threat-tagged" units were proposed to have an advantage over any

competing stimulus within the same network, increasing their chance of triggering RAM and subsequently leading to an attentional bias.

Despite revision, some limitations remain. The model's account of anxiety and attention seems limited, as only an attentional bias towards threat is emphasised. In contrast a vast body of evidence suggests that anxiety affects the breadth of attention, attentional scanning as well as distractibility and that all of these processes are affected by anxiety even in an absence of threat (e.g. Eysenck & Keane, 2013).

1.1.4 Mogg and Bradley's Cognitive-Motivational Perspective on Anxiety (1998)

Mogg and Bradley's (1998) Cognitive-Motivational Perspective on Anxiety identified two functional systems: "valence evaluation system" responsible for the appraisal of stimulus threat value, and "goal engagement system" responsible for directing behaviour towards external motivationally salient stimuli. They suggested that the subjective threat value assigned to a stimulus determines whether it will automatically capture attention, and considered biased threat evaluation to be the principal vulnerability factor for anxiety. Consequently anxiety is characterised by a tendency to assign high threat values to mild threatening stimuli and to subsequently allocate greater attentional resources to these anxiogenic stimuli. Specifically the model proposed a non-linear relationship between subjective threat value of the stimulus and attentional bias whereby alongside increased attention to high threat value stimuli, stimuli of low subjective threat value may be purposefully avoided; a notion supported by some studies (e.g. MacLeod et al, 1986; Mogg et al; 2000).

However, later research findings questioned the notion of attentional biases maintaining but not causing anxiety into question: most notably the studies by MacLeod et al. (2002) and Mathews and MacLeod (2002). These demonstrated that it is possible to modify patterns of attention to threat in non-anxious participants, and that such change alters affective responses to subsequent stress. MacLeod et al (2002) used a dot-probe task (see section 1.4.1 for extended discussion) to 'train' non-anxious participants either to adopt a biased attentional response toward threat words or an attentional bias away from threat. Attention bias modification modulated anxious responses to stressor, with anxiety being significantly greater in participants that were trained to attend to threat. This study provides evidence that selective attention to negative information has a causal effect on anxiety, and suggests a valuable treatment target.

1.1.5 Attentional Control Theory

Processing Efficiency Theory (Eysenck & Calvo, 1992) proposes that anxiety impairs the efficiency of the central executive by consuming resources that are required to attain a given performance level i.e. anxiety is characterised by poor performance efficiency, rather than poor effectiveness. The distinction between processing efficiency and performance effectiveness remains central to the Attentional Control Theory (ACT; Derakshan & Eysenck, 2009; Eysenck, Derakshan, Santos, & Calvo, 2007). Specifically ACT proposes that anxiety impairs attentional control, which is a key function of the central executive. This can be considered with reference to two attentional systems (e.g., Corbetta & Shulman, 2002; Posner & Petersen, 1990): a 'goal – directed' attentional system, influenced by expectation, knowledge, and current goals (involved in the top-down control of attention), and a 'stimulus – driven' attentional system, responding to salient or conspicuous stimuli (bottom-up control of attention) (Corbetta & Shulman, 2002). According to ACT, anxiety disrupts the balance between these two attentional systems by increasing the influence of the stimulus-driven system and decreasing the influence of the goal-directed system (e.g. by affecting the stimulus-driven attentional system via automatic processing of threat-related stimuli) (e.g. Fox, Russo, & Georgiou, 2005).

Evidence suggests that various executive functions are associated with the central executive: for example in a latent-variable analysis of executive tasks Miyake et al, 2000 identified inhibition, shifting, and updating functions. The inhibition function is thought to prevent task-irrelevant stimuli and responses from disrupting performance; the shifting function is thought to flexibly allocate attention to the task or currently most relevant stimulus; and the updating function is thought to update and monitor the information currently within working memory (vital for various short-term memory tasks). Miyake et al (2000) found that inhibition was used when resisting distractor interference and inhibiting responses, consistent with a role in executive control. ACT proposes that anxiety impairs processing efficiency due to reducing attentional control and increasing diversion of processing resources from task relevant to task-irrelevant stimuli.

Considerable evidence exists suggesting that anxiety impairs the inhibitory function (e.g. review by Derakshan & Eysenck, 2009). High-anxious individuals tend to be more susceptible to distraction than low-anxious individuals - (Pacheco-Ungietti, Acosta, Callejas, & Lupianez, 2010; PachecoUngietti, Lupianez, & Acosta, 2009). Impairment of the shifting function has also been observed in trait anxious individuals (e.g. Caselli, Reiman, Hentz, Osbourne, & Alexander, 2004; Ansari, Derakshan, & Richards, 2008; Wilson, Vine, & Wood, 2009). For example Ansari et al (2008) compared high and low anxious individuals on their performance on an antisaccade task

(see section 1.4.2) and found that high-anxious participants use the shifting function less efficiently than the low-anxious ones.

1.1.6 Neurocognitive mechanisms that underlie cognitive bias in anxiety

Recent years have seen a shift in the development of exclusively cognitive models to integrated neurocognitive models that incorporate recent evidence from neuroimaging. Bishop (2004; 2006; 2007) suggest that anxiety-related deficits in attentional control are mediated by reduced activity in the dorsolateral prefrontal cortex (DLPFC). Bishop's neurocognitive model of anxiety suggests anxiety could lead to threat related biases in selective attention due to interplay of hypofrontality (decreased activation of DLPFC) and a hyper activity of the amygdala.

Initial findings support both the increased amygdala response to threat distractors and the reduced recruitment of frontal control mechanisms (Bishop et al, 2004; Shin et al, 2001). Bishop (2009) used functional magnetic resonance imaging (fMRI) with a task that manipulated low and high perceptual load and required participants to discriminate between two target letters in the presence of task-irrelevant letter congruent or incongruent distractors. No difference in performance (target detection time) was found across groups between the congruent and incongruent distractor conditions in the high perceptual load-condition. However, only individuals with elevated trait anxiety had greater activation in the left DLPFC (associated with attentional control) in the incongruent than the congruent condition. In contrast, during low perceptual load the high-anxious group displayed reduced activation of the left DLPFC in the incongruent condition (low anxious individuals displayed an increased activation of the left DLPFC). These findings provide further evidence of deficits in attentional control in anxious individuals (Eysenck & Derakshan, 2011).

A two-stage competition model of attention has been proposed (e.g. Lavie, 2005), in which initial competition prevents distractors from being processed further under high perceptual task load, and at second stage, an active recruitment of control mechanisms prevents salient distractors from competing for further processing resources under low perceptual load. The findings of Bishop et al (2009) are in line with this account and consistent with findings linking high trait anxiety to diminished attentional control (Derryberry & Reed, 2002), and with suggestions of reduced recruitment of prefrontal mechanisms to aid regulation of aversive stimuli when cognitive resources are otherwise depleted (Kalisch et al, 2006). Incorporating these findings Bishop et al (2009) proposed a revised neurocognitive model of anxiety-related attentional biases (Figure 2). Here, attentional competition involves both early competition for perceptual resources and later competition for further processing, with trait anxiety modulating the processing at the

second ‘control’ stage of perceptual competition. The model also suggests that amygdala responsivity is primarily modulated by state anxiety whereas prefrontal recruitment is primarily influenced by individual differences in trait anxiety (Bishop et al, 2009).

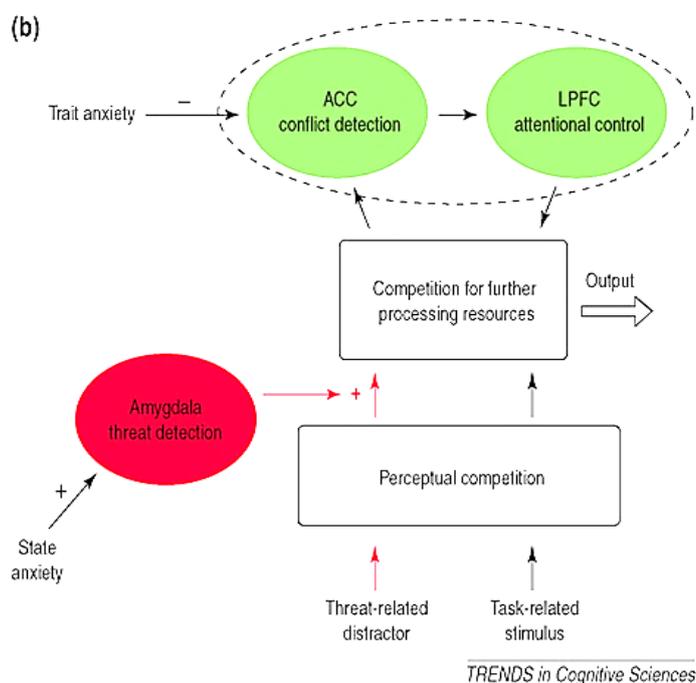


Figure 2 Neurocognitive model of anxiety (adapted from Bishop et al, 2006; 2009).

1.1.7 DLPFC – Amygdala interactions in anxiety

Serotonin (5-hydroxy-tryptamine, 5-HT) is thought to be involved in the regulation of mood, appetite, and sleep as well as in some cognitive functions, including memory and learning (King, 2009; Berger et al, 2009). Two of the 15 different serotonin receptors feature prominently in mood and anxiety disorders: 5-HT_{1A} and 5-HT_{2A} and these are two of the receptors affected by selective serotonin reuptake inhibitors (SSRIs) (Mann, Brent, & Arango, 2001). The 5-HT_{1A} receptor is widely expressed in the cerebral cortex, hippocampus, septum, amygdala, and raphe nucleus (e.g. Ito et al, 1999; de Almeida & Mengod, 2008). 5-HT_{2A} receptors are expressed primarily in the prefrontal, parietal, and somatosensory cortex. High concentrations of this receptor in the prefrontal cortex have been suggested to modulate cognitive processes, working memory, and attention (e.g. Aghajanian & Marek, 1999; Marek et al, 2001; Bortolozzi et al, 2005).

Intact serotonergic function facilitates cognitive and behavioural inhibition of emotional states and impulses, and low serotonergic function has been reported as a marker of a deficit in executive control (e.g. Carver, Johnson & Joormann, 2008). Individuals with altered serotonergic function are especially responsive to acute associative and affective cues in the environment (e.g.

Carver, et al, 2008) consistent with hypervigilance and corresponding deficits in attention control. Evidence supporting this notion includes investigations of the behavioural effects of serotonergic function using acute tryptophan¹ depletion to temporarily reduce serotonin. Such studies have found that tryptophan depletion impairs performance on cognitive tasks of executive processes such as reversal learning, memory, and attention tasks (e.g. Park et al., 1994; Riedel et al, 1999). Moreover, evidence that tryptophan depletion affects performance on behavioural and cognitive tasks that involve emotional stimuli also exists – for example one study saw acute tryptophan depletion to lead female participants to show slower processing of happy words (but not sad ones) in an affective go/no-go task (Murphy, et al 2002).

The 5-HT_{1A} and 5-HT_{2A} receptors co-locate on PFC suggesting that their functional interactions may mediate 5-HT effects on this circuit through top-down regulation of amygdala reactivity. Using multimodal neuroimaging in healthy volunteers, Fisher et al (2011) found that 5-HT_{1A} and 5-HT_{2A} receptors interact to shape serotonergic modulation of a functional circuit between the amygdala and PFC, suggested to help shape individual differences in sensitivity to threat and the related risk for psychopathology (Fisher et al., 2011).

1.1.8 Links with other models - De Raedt and Koster (2010)

Perseverative patterns of negative thinking (i.e. worry and to a lesser extent rumination) are a prominent feature of anxiety and comorbid conditions such as depression. Consequently neuropsychological models of depression can be useful in understanding the commonalities between the two disorders. De Raedt and Koster (2010) proposed such a conceptual framework, with deficits in attention control considered a central vulnerability to rumination and negative affect (see Figure 3). They proposed that during depressive episodes periods of hypercortisolism² impair the hypothalamic–pituitary–adrenal (HPA) axis, and sensitise it to stressors. As activity in DLPFC is mediated by serotonin metabolism (which is partially under control of the HPA axis) this can lead to decreased activity in DLPFC, which De Raedt and Koster (2010) link with prolonged activation of the amygdala in response to stressors in the environment. Impaired attenuation of amygdala activity through reduced frontal control leads to sustained negative affect. Decreased DLPFC activity also impairs inhibitory control and persists negative affect and stress reactivity. The relationship between this biological and cognitive conceptualisation is compatible with the findings that those with a tendency to ruminate show higher and prolonged amygdala activation

¹ Tryptophan is an essential amino acid in the human diet, which functions as a biochemical precursor to serotonin.

² Hypercortisolism is a state of excess production of the hormone cortisol.

when asked to temporarily increase their negative affect, as compared to controls (Ray et al. 2005).

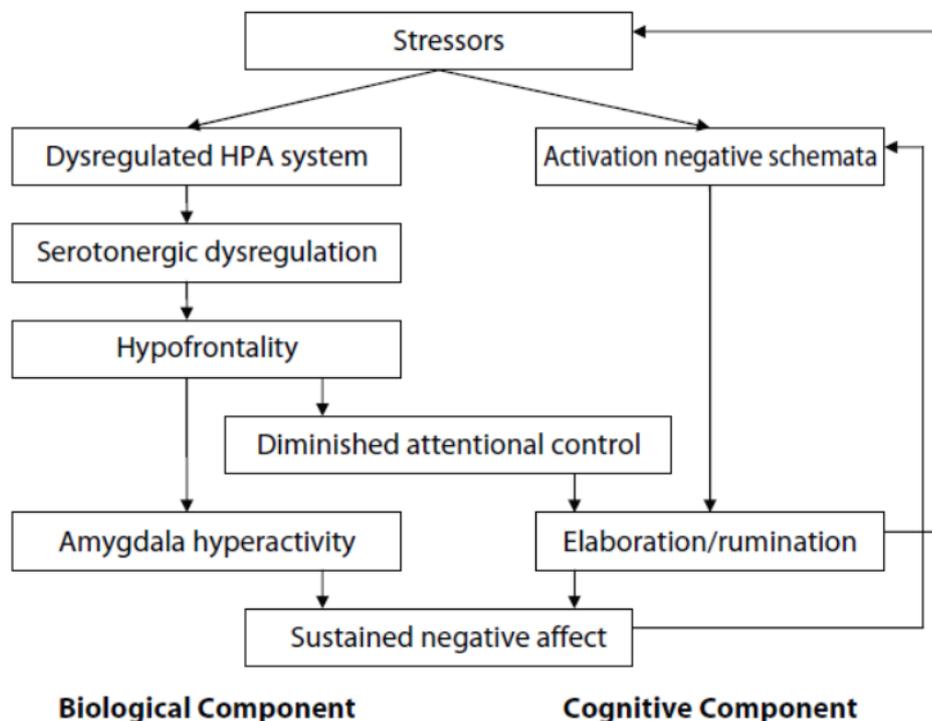


Figure 3 De Raedt and Koster (2010) model of depression and anxiety.

1.2 State versus Trait Anxiety

State and trait anxiety are thought to have different effects on executive functions, though interplay between the two also occurs. For example worry has been proposed as the component of state anxiety responsible for effects of anxiety on performance effectiveness and efficiency, but it has been emphasised that it is most likely to occur in individuals with high trait anxiety (see Eysenck, 1992 for a review). Evidence suggests that high trait anxiety is implicated in the impairment of the inhibiting function discussed earlier (section 1.1.5). Much of the relevant research has involved the emotional Stroop task (see Williams, Mathews, & MacLeod, 1996 for a review), which requires participants to name as rapidly as possible the colour of the presented neutral or threat-related words. The prediction is that there will be an emotional Stroop interference effect - the effects of anxiety in slowing colour-naming performance should be greater when the words are threat related. Because the inhibition of colour processing/naming is task relevant, slowed responses imply insufficient or compromised inhibition function. Mogg et al (1990) found that trait anxiety was positively associated with the magnitude of the emotional Stroop interference effect. Similarly Richards and French (1990) found the existence of the emotional Stroop interference effect with individuals high in trait anxiety having significantly

longer naming latencies for anxiety-related than for neutral words. Mogg and Marden (1990) found that high trait-anxious participants were slower at colour naming threat-related and emotionally positive words, suggesting that anxiety influences processing of all emotional words.

Threat-related attentional bias in anxiety has been investigated mainly in the context of trait (and rarely of state) anxiety and few studies have directly compared the effects of state and trait anxiety on attentional bias. Some studies examined whether state or trait anxiety scores were best correlated with attentional bias scores in clinically anxious individuals (e.g. Mathews & MacLeod, 1985; Mogg, Mathews, & Weinman, 1989). Others either experimentally induced state anxiety in non-clinical samples of varying levels with trait anxiety (e.g. Richards, French, Johnson, Naparstek, & Williams, 1992) or took advantage of naturally occurring stressful events (e.g. MacLeod & Rutherford, 1992). These studies yielded conflicting findings and led to various proposals as to the relative roles of state and trait anxiety in the attentional bias. For example, Broadbent and Broadbent (1988) suggested that the two interact, with the effect of state anxiety being substantially larger in individuals with high trait anxiety than in individuals with low trait anxiety. A somewhat different proposal is that whereas state anxiety and the attentional bias are positively correlated in individuals with high trait anxiety, they are negatively correlated in individuals with low trait anxiety (e.g. Egloff & Hock, 2001). Others suggested that both temporary stress (irrespective of trait anxiety) and enduring anxious personality characteristics (irrespective of state anxiety) increase attentional bias to threat (e.g. Mogg et al, 1990).

The relationship between different subtypes of anxiety and corresponding attentional biases may be better understood by acknowledging attention as a set of functionally and structurally independent networks rather than a unitary system (see Corbetta, Patel, & Shulman, 2008, and Posner, Rueda, & Kanske, 2007, for reviews). This is especially important when considering the use of experimental tasks (e.g. the Attention Network Test, see section 1.4.3) in measuring the attentional deficits in anxiety.

Posner and Petersen (1990) and Posner et al. (2007) distinguished three major attentional networks: namely, alerting, orienting, and executive control. Alerting is involved in maintaining an appropriate sensitivity level to perceive and process stimuli. It is thought to be related to activation of right frontal and parietal brain areas. The orienting network moves attention through space to attend to stimuli. It is thought to be associated with activations in the superior parietal lobe, frontal eye fields, and temporoparietal junction. The executive control network specializes in conflict resolution and is thought to be related to midline frontal areas, anterior cingulate gyrus, and LPFC. These frameworks are helpful in understanding the effects of trait and state anxiety on attentional processes. State anxiety increases as a direct consequence of current

situational factors; whereas trait anxiety is related to stable higher level processes, which operate across situations (i.e. top-down mechanisms). It is now been suggested that biases in anxiety may in fact be of a cognitive structural nature and could be observed under non-affective task conditions: for example Derryberry & Reed (2002) found trait anxiety scores and self-reports of attentional control to be negatively correlated. Rothbart, et al (2003) found a positive correlation between children's performance on emotionally neutral spatial conflict tasks and measures of voluntary control. More recently, Bishop (2009) reported neuroimaging evidence supporting this relationship, with trait anxiety related to a reduced recruitment of prefrontal structures critical in cognitive control mechanisms; and difficulties inhibiting distracting information were manifest even with non-affective distractors.

The frameworks and reviewed evidence provide a background for investigating the effects of trait and state anxiety on attentional network function (alerting, orienting and executive control) and the attentional biases present in anxiety, with the understanding that the biases may be cognitive in structure and present in non-affective task conditions.

1.3 Evidence of attentional control deficits and biases in anxiety

A number of experimental tasks have been used to test for attentional control deficits and biases in clinical and sub-clinical anxiety (e.g. Bar-Haim et al, 2007; Mogg et al, 1995): a meta-analysis of 172 studies found a reliable association between high anxiety and attentional biases (average effect size $(d) = 0.45$; bias consistent across a range of populations (adults and children), stimuli (words and pictures) and stimulus presentation durations (subliminal and supraliminal) (Bar-Haim, 2007). As anxious individuals preferentially allocate attention towards threatening compared to neutral stimuli across several experimental settings, this suggests this is not an artefact of a particular experimental procedure or task (Cisler & Koster, 2010). A range of studies utilising a variety of different tasks in both clinical and subclinical populations is summarised below.

1.3.1 Dot probe tasks

Attention bias is commonly measured with visual stimuli presented very quickly (e.g. 500 ms) in order to tap the initial stage of information processing. This task was adapted by MacLeod et al. (1986) from experimental cognitive psychology paradigms that indexed spatial attention from the speed of manual responses to visual probes (e.g. Posner et al., 1980; Navon & Margalit, 1983) that were presented in an attended vs. unattended regions of a visual display.

In a classic visual probe detection task/dot probe task (MacLeod et al, 1986) participants are briefly shown two stimuli comprising of emotionally valenced words or pictures (threat-related and neutral) at two different spatial locations on a screen. After the offset of these stimuli, a small target probe emerges at the location just occupied by one of the stimuli - either by the threatening stimulus (congruent presentation) or at the location of the neutral stimulus (incongruent presentation). Targets appear with equal probability at the location of threat and neutral stimuli, measuring the distribution of participants' attention (time needed to respond to the dot probe). An attentional bias towards threat is revealed when participants are faster to respond to probes that replace threat-related rather than neutral stimuli. The majority of probe detection studies found that anxious individuals do display an attentional bias to threat. Importantly conscious awareness of threat-related distractors is not necessary for attentional capture - anxious individuals were shown to orient to the position previously occupied by briefly presented backward-masked threat-related stimuli compared to a masked neutral stimuli, despite being unable to identify or even detect their occurrence (e.g. Mogg & Bradley, 1998).

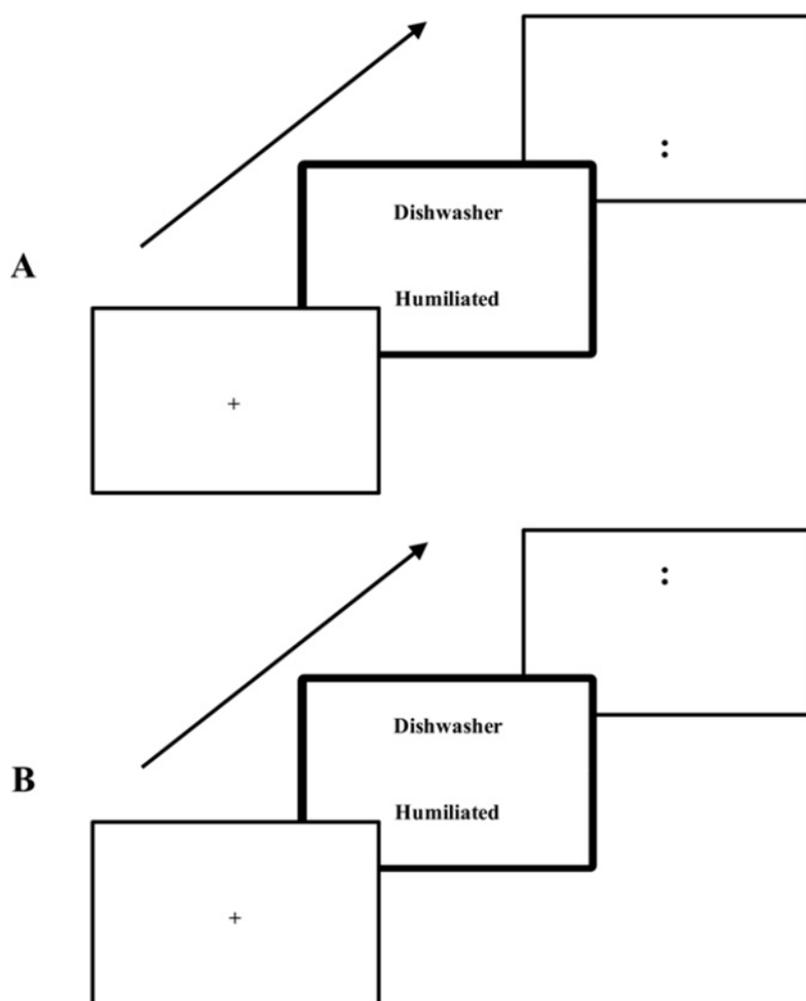


Figure 4 Classic dot-probe task (adopted from Hakamata et al, 2010)

1.3.1.1 Clinical Data

Macleod et al (1986) introduced the dot probe task as a novel paradigm, and demonstrated that clinically anxious participants consistently shift attention towards physically and socially threat-related words, resulting in reduced latencies for probes appearing in the vicinity of threat stimuli. Healthy controls displayed avoidance of threat words.

Bradley et al (1999) investigated attentional biases in GAD patients using the dot probe task but with more naturalistic stimuli – pictures of emotional facial expressions (threatening, happy and neutral). They additionally examined any potential biases for happy faces and assessed the time course of the attentional bias by manipulating the stimulus duration (500 ms vs. 1250 ms). GAD patients showed greater vigilance for threatening faces relative to neutral faces, compared with normal controls, an effect consistent across the two exposure durations. The study confirmed that GAD patients show a bias in selective attention to threat, relative to controls, and that this bias operates for naturalistic, non-verbal stimuli. In a later experiment, attentional biases were investigated in patients with GAD, Major Depressive Disorder (MDD) and healthy controls using eye tracking techniques during a dot probe task. All three groups performed similarly when responding to sad or happy faces, but on trials with angry faces only GAD patients exhibited an orientation bias, looking at the angry face first more often than the neutral face (Mogg, Millar & Bradley, 2000).

Dalgleish and colleagues (2003) compared anxious (GAD, PTSD) and depressed (MDD) children and adolescents on a range of cognitive tasks measuring attention, memory, and prospective cognition, with both threat-related and depressogenic stimulus materials. The results revealed that anxious participants exhibit a greater selective attentional bias for threat relative to depressogenic material with no such difference in the depressed sample. However, this bias was only exhibited on a dot-probe measure of attentional processing and not on other measures.

Mogg and Bradley (1995) reviewed the evidence of attentional biases in GAD patients and concluded that strong evidence of the attentional bias exists, observed primarily in dot probe and Stroop tasks. Anxious patients with predominant social worries have shown an enhanced bias for social threat (compared to physical threat) words on the visual probe task (Mogg et al, 1992). Other studies using the visual probe indicated that in GAD attentional biases are not specific to threat words but operate for general negative including depression-related words (e.g. 'sadness,' 'dejected,' 'worthless') (e.g. Mogg et al, 1995). Mogg and Bradley (2005) suggested this lack of content-specificity for threat stimuli might partly arise from the rather diverse content of worries in GAD.

1.3.1.2 Subliminal data

As with GAD patients, high trait-anxious individuals tend to respond faster to probes previously occupied by threatening, rather than neutral stimuli (e.g. Mogg & Bradley, 1998). Fox et al (2002, experiment 2) found that individuals with high trait anxiety had a larger attentional bias index for masked fearful than for unmasked fearful faces in the dot probe task, whereas there was no difference for the happy faces. For low anxious individuals, there was no difference for either the fearful or the happy index scores between the masked and the unmasked conditions.

In a more recent study Eldar et al (2010) used a dot probe task measuring attentional bias towards both threat (angry) and positive (happy) face stimuli in a sample of high and low anxious individuals. Participants were selected based on their scores on the trait scale of the State-Trait Anxiety Inventory (STAI-T) (Spielberger et al, 1983). The anxious group consisted of participants scoring highest on trait-anxiety (mean score similar to those among clinically anxious patients (e.g. Fisher & Durham, 1999; Yong-Ku et al, 2009) and the non-anxious group comprised those scoring lowest. The results confirmed an attention bias towards threat among anxious participants, but not among non-anxious participants. No bias to positive faces was found in either group.

1.3.2 Antisaccade task

In the antisaccade task (see Figure 5) participants are instructed to make a saccadic eye movement to the opposite side of a visual cue presented to the left or the right of the fixation point as rapidly as possible. Two processes are needed for the anti-saccade task: suppression of the automatic pro-saccade, and inversion of the stimulus vector into the correct saccade vector (see review by Kristjansson et al, 2001). The latency of the first saccade to the correct side is one of the main dependent variables of interest. There is also a control task (the pro-saccade task), in which the instructions are to fixate the cue when it appears. Anti-saccades have longer latencies than pro-saccades and participants are more likely to make errors on the anti-saccade trials as opposed to pro-saccade trials (regardless of anxiety levels). These errors usually consist of a rapid saccade to the target, which is often corrected within a short latency by a second saccade away from the target. The use of the antisaccade task to investigate the effects of anxiety on attentional control has been suggested by Miyake et al (2000). According to ACT, adverse effects of anxiety in terms of latency of the first correct saccade should be present with the antisaccade task but not the pro-saccade task.

The antisaccade task provides two distinct performance measures: *performance effectiveness* measured by the proportion of trials where participants are unable to make the antisaccade (i.e.

error rate), and *processing efficiency* measured by the time required to successfully perform correct saccades (Kristjansson et al, 2001; Eysenck et al, 2007).

Ettinger et al. (2008) found evidence that DLPFC and ventrolateral prefrontal cortex activation were the strongest predictors of performance accuracy, consistent with neurocognitive models that implicate these regions in anxiety-related deficits in attentional control.

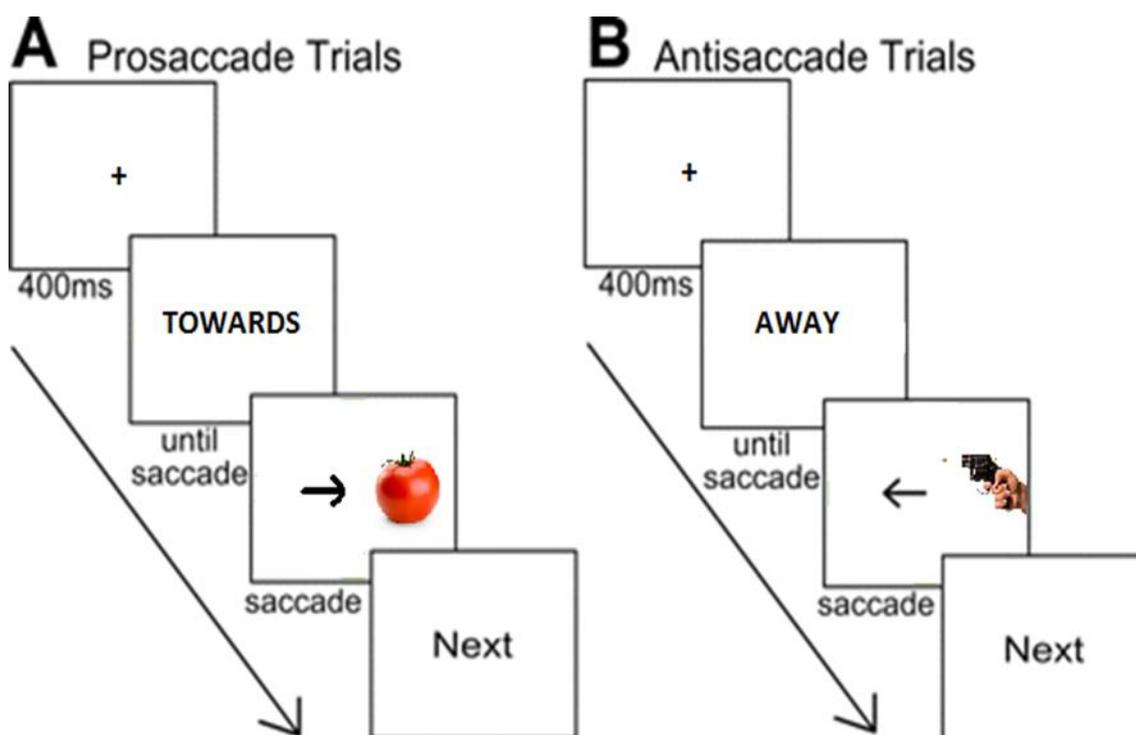


Figure 5 The Antisaccade Task

1.3.2.1 Clinical data

Two studies with clinically anxious adolescents provide evidence supporting the existence of attentional bias to threat using antisaccade task (Jazbec et al, 2005; Hardin et al, 2009). Jazbec et al (2005) compared the performance on an incentive modified antisaccade task with three contingency contexts (monetary gain = reward condition), monetary loss = punishment condition, and no incentive = neutral condition) between healthy youth, clinically anxious youth (GAD and GAD comorbid with other anxiety disorders e.g. separation anxiety disorder, social and specific phobias) and youth with MDD. An impaired performance on the antisaccade task in clinically anxious youth was found characterised by higher error rates on all three congruency trials as well as longer latencies of incorrect antisaccades in both the punishment and reward conditions as compared to the healthy sample.

Hardin et al (2009) conducted a study using the Incentive Emotion Antisaccade Task with two explicitly presented incentive conditions (Reward, No Reward) in adolescents diagnosed with an anxiety disorder (Social Phobia, GAD, and comorbid GAD and MDD; medication free) and age-matched healthy adolescents. Each incentive condition was paired with three face emotion conditions (happy, fearful, and neutral). Emotion faces appeared concurrently with the incentive cues, but transferred no task-related information. Anxious adolescents produced shorter antisaccade latencies in the fear condition than in the neutral or happy conditions, when compared to healthy adolescents.

1.3.2.2 Subclinical data

Several studies have shown that individuals with elevated levels of trait anxiety make slower antisaccades than low anxious control participants (e.g. Derakshan et al, 2009a; Ansari & Derakshan, 2011; Ansari et al, 2008; Reinholdt-Dunne et al, 2012). Reinholdt-Dunne et al, (2012) conducted antisaccade and pro-saccade tasks (angry, fearful, happy, and neutral faces) in healthy adults with high and low trait anxiety and found that high-anxious participants were slower to look away from angry faces compared with low-anxious individuals. This bias was not found for fearful or happy faces and was not related individual differences in self-reported attention control.

Derakshan, et al (2009) used the antisaccade task (neutral oval shape as a cue) in a group of healthy individuals with low (<13 on STAI-Trait) and high (>16 on STAI-Trait) anxiety and found that adverse latency of the first correct saccade was impaired in the high anxious group on the antisaccade but not the pro-saccade task. In a second study, Derakshan et al. (2009) used angry, happy, and neutral faces as cues and observed anxiety-related deficits in antisaccade performance, particularly in response to angry cues. Similarly using emotional cues (negative and neutral pictures) on the antisaccade task Garner et al (2009) found that high-anxious individuals made significantly more eye-movement errors than low-anxious individuals on antisaccade (but not prosaccade) trials regardless of cue type. Together these findings recommend the antisaccade task for characterising attentional biases in anxiety (for a review see Ainsworth & Garner, 2013).

1.3.3 Attentional Network Test

The Attention Network Test (ANT) was developed to allow for an independent assessment of the efficiency of the 3 attentional networks proposed by Posner and Petersen (1990) (see section 1.3), with a quick and easy computerized task (Fan, McCandliss, Fossella, Flombaum, Posner, 2005; Fan, McCandliss, Sommer, Raz and Posner, 2002).

The ANT is a combination of a flanker task with arrows (Eriksen & Eriksen, 1974) and a cued reaction time task (Posner, 1980). It requires indicating the direction of a central arrow, which is flanked by 4 arrows (2 on each side, for example $\leftarrow\leftarrow\rightarrow\leftarrow\leftarrow$) with flanker arrows either pointing in the same direction as the target arrow (congruent condition) or in the opposite direction (incongruent condition). As a reaction time task, the ANT provides two measures of performance: *response time* (RT) and *error rate* (ER).

1.3.3.1 Clinical data

Pacheco-Unguetti et al (2011) investigated the relationship between pathological anxiety and attentional mechanisms using a modified version of the ANT, with clinically anxious patients and healthy controls. All patients met the diagnostic criteria for an anxiety disorder (the majority diagnosed with GAD). Significant differences on the orienting and executive control networks were found between the clinical and non-clinical groups, which suggest that anxiety disorders are related to both reduced effectiveness of the executive control network and difficulties in disengaging attention from invalid cues, even when using emotionally neutral information. More recently patients with comorbid GAD and MDD were found to exhibit a faster orienting response on the ANT compared with MDD patients (Han et al, 2012).

1.3.3.2 Subclinical data

Pacheco-Unguetti et al (2010) investigated the effects of anxiety on attention network function in a healthy sample (high and low trait and state anxiety) in two separate studies using a modified version of the ANT. The first study indicated that high-trait-anxiety was associated with a general deficit in executive control, supporting previous findings by Pacheco-Unguetti, Lupiáñez, and Acosta (2009). Alerting and orienting networks did not differ between high and low trait anxiety groups. The second study revealed increased alerting and orienting function in state anxious individuals (but not the executive control). These findings are consistent with models that highlight broad hypervigilance to contextual cues in state anxiety (perhaps an adaptive response), but more problematic deficits in executive attention control in individuals with elevated trait anxiety.

Reinholdt-Dunne et al (2009) found that participants who reported high trait anxiety in addition to poor self-reported attention control were characterised by greater cognitive interference by emotional faces (including angry faces), compared to neutral faces (i.e. poorer executive control). Furthermore experimental methods that increase anxiety (e.g. using the 7.5% CO₂ model also modulate ANT performance (see section 1.7.1).

In sum, the reviewed evidence suggests the existence of deficits in executive control in highly trait anxious and clinically anxious participants.

1.3.4 Evidence from other tasks

Studies using dot-probe, antisaccade and ANT tasks provide a substantial body of evidence for the existence of an attentional bias in anxiety. Other paradigms and techniques such as measuring event-related potentials (ERP³) or using neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have also been used to further support the notion of attentional bias in anxiety.

1.3.4.1 Matching Task (Vuilleumier et al, 2001)

Two black and white photographs were situated in either the north–south or east–west positions of a cross-format display that comprised four photographs arranged around a central fixation point. All four photographs were presented concurrently but at the start of each block, participants were cued to attend selectively to either the two vertically arranged or two horizontally arranged positions, while being asked to ignore the alternative two locations throughout the block. Within each trial, either the two attended or the two unattended locations were occupied by the photographs of two faces (either fearful or neutral), and the two remaining locations by photographs of two houses (each trial could be classified as faces-attended or faces unattended). Thus four conditions existed: face attend-fearful, face attend-neutral, face unattend-fearful, and face unattend-neutral, with trial types and pair identities (i.e, pictures being the same or different e.g neutral + neutral or neutral + fearful). The task requires the participants to indicate, as accurately and rapidly as possible, whether the two stimuli at task-relevant locations were the same or different, recording latency and accuracy.

³ Event-related potentials (ERPs) are small voltages generated in brain structures in response to specific events or stimuli (Blackwood & Muir, 1990). They are EEG changes that are time locked to sensory, motor or cognitive events that provide safe and non-invasive approach to study psychophysiological correlates of mental processes (Sur & Sinha, 2009). Two aspects of ERP particularly implicated in anxiety are P300 and late positive potential (LPP). For the P300 wave for auditory stimuli, the latency range is 250-400 milliseconds for most adult subjects. The latency is usually interpreted as the speed of stimulus classification resulting from discrimination of one event from another with shorter latencies indicating superior mental performance. Greater attention produces larger P3 waves (Sur & Sinha, 2009). ERPs elicited by threat-relevant stimuli support the existence of an attentional bias, showing larger amplitude of P300 and slow waves in response to fear-related words or pictures in subjects with high-trait anxiety or anxiety disorders when compared with healthy controls (De Pascalis et al., 2004).

1.3.4.1.1 Clincial data

MacNamara and Hajcak (2010) used the Matching Task in patients with GAD and in healthy controls whilst measuring participants' late positive potentials (LPP⁴). The participants viewed briefly presented pairs of aversive and neutral pictures, presented to the left and right of, as well as above and below, fixation on and were asked to indicate whether the horizontal or vertical image pairs were the same or different. Aversive pictures presented in unattended locations were associated with more errors overall, and this effect was larger in GAD than in control participants. Moreover, aversive targets elicited larger LPPs across all participants; again this difference was larger in GAD than control participants when distracters were neutral. This suggests that threatening stimuli presented in both target and distracting spatial locations have a greater impact on GAD than on healthy participants.

1.3.4.1.2 Subclinical data

MacNamara and Hajcak (2009) used the Matching Task whilst measuring LPPs in a group of healthy high and low state anxious individuals. Participants were required to indicate whether two of four presented pictures (either neutral or aversive) (left, right, top and bottom of the screen) on each trial were the same or different. Aversive images presented in unattended locations resulted in increased error rate and reaction time. The LPPs was larger only when aversive images were presented in attended locations, and this was positively correlated with self-reported state anxiety.

Bishop, Duncan, Brett and Lawrence (2004) suggested distinct roles for rostral ACC and LPFC in governing the processing of task-irrelevant threat-related stimuli, modulated by state anxiety in the Matching Task during fMRI acquisition. Participants attended to pictures of two houses, deciding whether they were identical or not and the faces were task-irrelevant distractors. When initial trials all contained threatening distractors, low-anxiety participants showed increased recruitment of DLPFC and VLPFC, as compared with high-anxious participants, providing evidence of hypofrontality in anxiety.

⁴ LPP is a scalp-recorded ERP observed by 300 ms after stimulus presentation, which extends throughout the duration of stimuli presentation and indexes increased sustained attention to emotional stimuli (e.g. Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Foti, Hajcak, & Dien, 2009; Hajcak, MacNamara, & Olvet, 2010b; Hajcak & Olvet, 2008; Schupp, Junghöfer, Weike, & Hamm, 2003). Evidence suggests that LPP becomes larger with increasing state anxiety (e.g. MacNamara & Hajcek, 2009).

1.3.4.2 Visual search task

Visual search is a perceptual task requiring attention. It typically involves an active scan of the visual environment for a target among distractors. For example Lavie and Cox (1997) asked participants to ignore an irrelevant peripheral distractor presented outside the search area whilst searching for one of two target letters (X or N) among other non-target letters in an array. The task can also be used with pictorial stimuli.

1.3.4.2.1 Subliminal data

Bishop, Jenkins, and Lawrence (2007) required participants to search for an X or an N in a six-letter string under either low or high perceptual load (all letters the same; or target letter embedded among non-target letters; respectively), the letter strings being superimposed on a background of neutral or fearful faces. fMRI was performed during the task and indicated similarities in brain activations under high perceptual load, regardless of anxiety levels and valence of distractor faces; but significant differences under low-perceptual load: with fearful face distractors, high state anxiety was associated with a heightened response in the amygdala, whereas high trait anxiety was related to a reduced prefrontal response. High state anxiety is thus suggested to activate regions associated with the assessment of the valence of facial expression, whereas high trait anxiety with a reduced activity in regions associated with control processes.

More recently Matsumoto (2010) used the visual search tasks to investigate two different components of attentional bias to threat: engagement and disengagement of attention from an angry face. Two main results were found. First, reaction times were slower in detecting the absence of a discrepant face in the all angry-display conditions rather than other expression conditions; however, there was no difference between anxiety groups. Second, the difference in search efficiency for the angry versus happy target was significant within the high-anxiety group but not within the low-anxiety group. The results suggest that the detection process for angry faces is more efficient for highly anxious people. On the other hand, the time to disengage attention from angry faces was not associated with anxiety level.

A later study with no emotional material replicated a reduced prefrontal activation in high trait anxiety (Bishop, 2009), thus suggesting that trait anxiety, but not state anxiety, may be associated with cognitive control.

1.3.4.3 Resting state fMRI (no task)

Resting state fMRI is a method of functional brain imaging that can be used to evaluate regional interactions that occur when a subject is not performing an explicit task (Biswal, 2012; Buckner,

Krienen & Yeo, 2013). Resting brain activity is observed through changes in blood flow in the brain which creates a blood-oxygen-level dependent (BOLD) signal that can be measured using fMRI. Because brain activity is present even in the absence of an externally prompted task, any brain region will have spontaneous fluctuations in BOLD signal. The resting state approach is useful to explore functional organization in neurological and psychiatric conditions.

1.3.4.3.1 Subclinical data

Kim et al (2011) examined the functional connectivity between amygdala and prefrontal brain regions. At rest, amygdala activity was positively correlated with activity in ventral mPFC regions, and negatively correlated with activity in dorsal mPFC regions. Across the whole brain, anxiety scores predicted resting-state functional connectivity between the amygdala and only two regions—the dmPFC and vmPFC. Resting-state functional connectivity between the right amygdala and the dmPFC was positively correlated, and between the right amygdala and the vmPFC negatively correlated, with state anxiety. This suggests that the negative correlation between amygdala and dmPFC activity observed in all subjects at rest was only preserved in subjects with lower levels of state anxiety, with this pattern of connectivity no longer evident in those with higher levels of state anxiety.

Similarly, the positive relationship between activity in the amygdala and vmPFC observed in all subjects at rest was compromised in subjects with higher levels of state anxiety.

These data show that amygdala–mPFC connectivity at rest reflects normal individual differences in anxiety and provides further evidence that prefrontal regions, crucial in attention control, are less active in anxiety.

1.4 Current interventions for anxiety

Currently first line treatments for GAD are either Cognitive Behavioural Therapy (CBT) or selective serotonin reuptake inhibitors (SSRI)s. CBT has been one of the most repeatedly and extensively evaluated psychological treatment interventions for GAD (Rygh & Sanderson, 2004). CBT targets cognitive and behavioural mechanisms (e.g. biases in attention, threat appraisal and uncertainty), whereas SSRIs rapidly block serotonin (5-HT) reuptake.

1.4.1 Cognitive Behavioural Therapy (CBT)

CBT addresses the role of dysfunctional thinking in patients' behaviour which in GAD typically includes: patient self-monitoring of worrying or related symptoms; cognitive restructuring (including evaluating and reconsidering interpretive and predictive thoughts/worries); relaxation

training; and rehearsal of coping skills (Borkovec & Ruscio, 2001). Patients may be asked to monitor their symptoms of anxiety along with situational factors that lead to increased anxiety, which helps the patients recognize triggers of anxiety and patterns of maladaptive thinking (Kavan et al, 2009). CBT focuses on teaching patients to challenge unwarranted worrying and to replace these thoughts with problem-solving strategies. Patients may also be taught the use of self-calming techniques (e.g. deep breathing, relaxation) to reduce physiologic arousal and enhance their sense of control over their symptoms (i.e. to reduce state anxiety).

CBT can be an effective treatment for GAD (e.g. Barlow, Rapee & Brown, 1992; Ladouceur, Dugas, Freeston, Leger, Gagnon & Thibodeau, 2000). Early studies comparing active forms of CBT with non-directive treatment and/or control groups report significantly greater symptom improvements for the active CBT groups (e.g. Barlow, Rapee & Brown, 1992; Blowers, Cobb & Mathews, 1987; Lindsay, Gamsu, McLaughlin, Hood & Espie, 1987). Specific versions of CBT designed to tackle intolerance of uncertainty, erroneous beliefs about worry, poor problem orientation and cognitive avoidance were found to produce large (77%) improvements in those symptoms compared to a delayed-treatment condition (Ladouceur, Dugas, Freeston, Leger, Gagnon & Thibodeau, 2000). CBT has also been suggested to be significantly more effective than both applied relaxation and a non-directive therapy at a 12 month follow up (Borkovec and Costello, 1993).

A number of meta-analyses of CBT in anxiety have found large effect sizes (e.g. Otte, 2011; Olatunji et al, 2010; Butler et al, 2006; Deacon & Abramowitz, 2004). However, these meta-analyses are not without limitations, in particular, by including studies varying greatly with respect to control procedures (ranging from waitlist, alternative treatments, placebo interventions or no control group; evaluated with or without randomization). A meta-analysis by Hoffman et al (2008) was limited to studies of randomized placebo-controlled trials (with the psychological placebo having to involve interventions to control for nonspecific factors). Overall, 27 studies met inclusion criteria (7 SAD, 6 PTSD, 5 panic disorder, 4 acute stress disorder, 3 OCD, and 2 GAD) with CBT achieving a medium effect size for GAD (Hedges' $g^5 = 0.51$), although it is difficult to draw meaningful conclusions with such a small sample size of GAD studies. A Cochrane meta-analysis aggregating psychological treatments of GAD in 13 studies (Hunot et al, 2007) suggests that CBT was more effective than a wait list control in achieving clinical response at post-treatment, however when examined against supportive therapy (nondirective therapy and attention-placebo conditions) significant difference in clinical response was no longer found. A meta-analysis by Stuart and Chambless (2009) included 56 studies (17 panic disorder, 11 SAD, 11 GAD, 11 OCD, and

⁵ Hedges' g is a variation of Cohen's d taking into account small sample sizes.

6 PTSD) and assessed how CBT treatment works in less well-controlled real-life settings. CBT was defined broadly and included any treatment with cognitive, behavioural (e.g. exposure), or a combination of components. For effectiveness of CBT in GAD there was a large pre- to post-treatment effect size. In sum, support for efficacy and effectiveness of CBT in treatment of GAD exists, when compared to no treatment or another psychological treatment.

When compared with pharmacological interventions, a meta-analysis of controlled studies utilising CBT and/or pharmacotherapy with GAD found no statistically significant differences in terms of effectiveness at post treatment, however CBT was found superior in terms of long term effects, with gains of treatment being maintained with CBT but attenuated following medication discontinuation (Gould, Otto, Pollack & Yap, 1997). A more recent meta-analysis (Roshanaei-Moghaddam et al, 2011) which included 21 anxiety and 21 depression studies comparing medication to CBT, found that in panic disorder CBT was superior to medications, whereas for SAD there was an advantage for medication: the effect for GAD was challenging to evaluate as only a single study was included. Pooling anxiety disorder and depression studies, the overall comparison of the relative difference between anxiety and depression in effectiveness for CBT versus pharmacotherapy suggested a non-significant advantage for CBT in anxiety versus depression.

1.4.1.1 Limitations

Despite being a first line treatment, CBT has limitations. Some patients do not have access to CBT and waiting lists are also typically long. Those with access may find it demanding as it requires patients to engage with and comprehend complex concepts (which is why perhaps CBT is more efficacious in those with higher socioeconomic status, and higher levels of education (e.g. Arch & Craske, 2009; Newman, 2000). A substantial number of GAD patients (30-40%) do not respond to CBT (e.g. Rygh & Sanderson, 2004) and remission rates after CBT tend to oscillate around 50% (Ballenger, 2004; Barlow, Gorman, Shear & Woods, 2000; Cartwright-Hatton, et al, 2004).

An active pursuit for more efficacious treatments for GAD remains an imperative. To tackle these problems a guided internet-delivered CBT for GAD has been developed (e.g. Paxling, Almloev, Dahlin, Carlbring, Breitholz, Eriksson & Andersson, 2011). Paxling et al (2011) randomized participants to either an 8-week treatment group (self-help program based on CBT and applied relaxation) or a wait-list control group and found that the treatment group showed significant improvement compared with the control group on all outcome measures with large effect sizes both within the treatment group and between the groups favouring the treatment. Results at 1- and 3-year follow-up indicated that treatment results improved or were maintained, suggesting that Internet-delivered CBT can be a useful tool in reducing symptoms related to GAD.

CBT as a technique lacks a means of directly manipulating attentional biases present in anxiety, as CBT protocols focus primarily on training in relaxation techniques, fear extinction via systematic and graded exposure to threat, and conscious challenging of negative interpretive and memory biases (e.g. Bar-Haim, 2010). Thus Attentional Bias Modification Training (AMBT) (see section 1.6.1) stemming from established experimental data on threat-related attentional biases in anxiety (for a review see Bar-Haim, et al, 2007), presents a novel alternative treatment. It utilises a computer-based attention training protocol to implicitly modify the biased attentional patterns in anxious patients and subsequently lead to a reduction in their anxiety levels (Bar-Haim, 2010).

1.4.2 SSRI

Selective serotonin reuptake inhibitors (SSRIs) have emerged as the first-line pharmacological treatment for patients with GAD. SSRIs are recommended by the National Institute for Health and Clinical Excellence (NICE) for the treatment of GAD, especially in patients for whom non-pharmacological treatment attempts have failed (NICE, 2013). They provide a modest-to-moderate reduction in anxiety in GAD (NICE, 2013) with many studies suggesting their superiority over placebo (e.g. Kapczinski et al, 2003; Birmaher et al, 2003; Lenze et al, 2011 Davidson et al, 2004; Stocchi et al, 2003; Liebowitz et al, 2003).

SSRIs mechanism of action is via rapid blockade of serotonin (5-HT) reuptake. However weeks of treatment are required before the onset of their therapeutic action, which has been suggested to be the result of presynaptic and postsynaptic adaptive mechanisms secondary to reuptake inhibition (e.g. Celada et al, 2004). At the neuronal level when the patient is treated with an SSRI, serotonin released from a raphe neurone into the synapse in a projection area (e.g. the cortex) can act on a number of postsynaptic 5-HT receptors. By also acting on the presynaptic 5-HT receptors and 5-HT_{1A} receptors on the cell body, serotonin can inhibit the firing rates of the cells thus decreasing the release of further serotonin. SSRIs block the reuptake of serotonin into the presynaptic nerve terminal via the serotonin uptake site, thus increasing the synaptic concentration of serotonin, resulting in increased activation of the presynaptic inhibitory receptors and decreased firing of the serotonergic neurone (Nutt et al, 1999).

During SSRI treatment, there is an initial inhibition of raphe cell firing. After about 3 weeks, however, the firing rate gradually increases, and the synaptic concentration of serotonin in the cortex reaches therapeutic levels.

The effects of SSRIs extend beyond self-report measures of anxiety as they can also reduce cortisol levels both at peak and total levels (e.g. escitalopram; Lenze et al, 2011). Moreover a distinct advantage of the use of SSRIs is their capacity to treat comorbid psychiatric disorders such

as depression, OCD, panic disorder, and PTSD that often accompany GAD and social phobia (Ballenger et al., 1998, 2001).

Recently Bandelow et al (2015) conducted a meta-analysis comparing the efficacy of pharmacological, psychological and combined treatments for three common anxiety disorders: panic disorder, GAD and social phobia. The authors calculated the pre-post as well as the treated versus control effect sizes for all evaluable randomized-controlled studies (n=234), which in total included 37 333 patients. Medications were associated with significantly higher average pre-post effect sizes than psychotherapies (e.g. 2.25 for SNRIs, 2.15 for benzodiazepines and 2.09 for SSRIs vs 1.56 for mindfulness therapies, 1.30 for CBT and 1.11 for Internet therapies). There was a large effect size for CBT/drug combinations (2.12). In contrast effect size for psychological placebos was 0.83 and 0.20 for waitlists. Medications emerged as more effective than psychotherapies.

1.4.2.1 Limitations

Some of the more common adverse events associated with SSRI use include nausea, sexual dysfunction, agitation, weight gain, and insomnia and although these effects tend to be mild, some may be mistaken for worsening anxiety and lead to non-adherence (Kavan et al, 2009). A large proportion of patients also experience worry related to drug dependency. These problems limit the effectiveness of pharmacological treatments in clinical practice (Baldwin et al, 2010). It can be difficult to reliably predict which patients will respond well to the SSRI treatment and which will have only a limited response, which can make response rates disappointing (Baldwin et al, 2010). Moreover the pharmacological treatment does not target any of the attentional biases present in GAD.

1.5 Can we directly target neurocognitive mechanisms underlying anxiety?

As the reviewed literature suggests, there are a number of cognitive and attentional biases present in anxiety, and corresponding hyperactivation of the amygdala and hypoactivation of the PFC. Current first line treatments for GAD have limitations and don't directly target neurocognitive mechanisms. The question remains, can these mechanisms and biases be reliably targeted, and subsequently, does successful targeting of these biases result in reductions in anxiety?

1.5.1 ABMT

Attentional Bias Modification Training (ABMT) is a potentially successful therapeutic intervention for anxiety that targets dysfunctional attentional bias in anxiety. Using a modified dot probe task MacLeod, et al (2002) were the first to demonstrate that the attentional bias can be induced in a healthy population. ABMT is a modified version of the classic dot probe task (see section 1.4.1), where the probability of the probe appearing at the location previously occupied by neutral stimuli is not 50% (like in the classic version of the task) but a 100%. Over time this contingency encourages participants to attend to neutral stimuli and develop a 'natural' propensity to reduce attention to threat.

ABMT (unlike CBT) does not explicitly ask anxious individuals to consciously exert top-down effortful attention control, but rather implicitly targets early, automatic and sometimes unconscious attentional biases through computer-based attention training protocols.

There is a growing literature suggesting that ABMT is effective in its modification of selective attention to threat, and in subsequently reducing levels of anxiety vulnerability (e.g. Macleod and Clarke, 2013) (see Table 1.1 for examples). MacLeod et al (2002) used the dot-probe task to train non-anxious participants either to adopt a biased attentional response toward threat words or an away from threat, and found the two groups of participants developing differentially biased attention responses in accord with the assigned threat-target contingency. This differential attention bias did not result in an immediate change in anxiety following training (low baseline trait anxiety across groups), however it led to a group difference in anxiety vulnerability to a subsequently induced stress using a standardized stress task. Both groups responded to the task with elevation in state anxiety; however the magnitude of this elevation was significantly greater in those exposed to the "attend threat" as compared to "attend neutral" condition.

Amir, Weber, Beard, Bomyea, and Taylor (2008) in a single dot-probe session with socially anxious students either trained them to attend away from facial expressions of disgust or to perform a standard probe task; and found that those trained to attend away from disgust stimuli showed significantly less attention bias after training (as compared standard task). Moreover this was accompanied by lower levels of anxiety in response to a subsequent stress-inducing public-speaking challenge. These results were replicated by Heeren, Reese, McNally, and Philippot (2012), who extended these findings to report that the post-training attentional bias change mediates changes in skin conductance reactivity to an impromptu speech. These results support the effectiveness of ABMT in both reducing the attentional threat bias and in reducing physiological features of anxious arousal.

ABMT has been examined in multiple clinical samples (see Clarke et al, 2014 for a review). Three early studies provided compelling evidence that ABMT can attenuate clinical symptoms of anxiety. In a randomized controlled trial, Schmidt Richey, Buckner, and Timpano (2009) assigned patients with generalized social phobia to either eight sessions of ABMT training, aimed to reduce vigilance to threat or to a control condition, and found that post-intervention, 72% of those in the ABMT condition no longer met diagnostic criteria for social phobia, compared to only 11% in the control condition. At follow-up assessment four months later, the therapeutic gains were maintained, with 64% of the participants in the ABMT condition classified as remitted, compared to only 25% of those in the control condition. These results were replicated by Amir and colleagues (2009a) using identical procedure as Schmidt et al (2009) and with similar results (50% in the ABMT and 14% in the control group no longer met diagnostic criteria). Amir, Beard, Burns, and Bomyea (2009b) conducted a study with GAD patients, randomly allocated to either an ABMT condition designed to induce avoidance of threat words or to a control condition. This dot-probe training entailed 160 trials of threat-neutral word pairs per session, administered in eight sessions across a four-week period. Following training 58% of patients in the ABMT condition no longer met DSM-IV diagnostic criteria for GAD compared to only 17% of the patients in the control condition. The clinical efficacy of the attentional training procedure was further supported by improvements on a range of clinician-delivered and self-report measures of anxiety.

Heeren et al (2011) have raised an issue regarding the differentiation between training to disengage from threat and training to engage towards non-threat. Patients with social phobia were randomly assigned to one of four training conditions: (1) disengagement from threat, (2) engagement towards non-threat, (3) disengagement from threat and re-engagement towards non-threat, and (4) a control condition. Data revealed that training to disengage from threat reduces behavioural indices of anxiety whereas engagement towards non-threat faces did not. Consequently training attention away from threat (i.e. greater disengagement), rather than towards benign stimuli is potentially a more promising therapeutic target.

However some studies have not demonstrated clinical benefits of ABMT under specific conditions. The first of these was the randomised control trial reported by Carlbring et al, (2012), in which ABM failed to modify both the biased attention and the anxiety symptoms in a group of socially anxious individuals over the Internet. A number of other studies that have delivered intended attentional bias modification tasks have neither successfully modified biased attention nor impacted on anxiety levels (e.g. Boettcher et al, 2012; Boettcher et al, 2013; Neubauer et al, 2013 - SAD; Schoorl et al, 2013 – PTSD). Moreover, it is not the case that patterns of biased attention were successfully altered but this failed to influence measures of anxiety - instead, these unsuccessful trials did not alter patterns of selective attention in the first place. Hence, these

studies should be regarded as manipulation failures, rather than evidence against the potential therapeutic value of attentional bias modification. It has been suggested that the fact that studies which have failed to modify selective attention have also failed to modify emotional vulnerability provides reassurance that the theoretical basis for ABM is sound (Clarke et al, 2014).

Recently Clarke et al (2014) reviewed 42 ABM studies, of which 29 included measures of attentional bias change and emotional vulnerability, and are therefore able to inform the link between this proposed causal mechanism and its emotional impact. Of these 29 studies, 26 are consistent with the relationship described above – successful training of an attentional bias produces subsequent changes in behavioural/mood measures ($n = 16$), whereas failure to train attentional bias does not alter mood ($n = 10$). Of the three remaining studies, one successfully modified attentional bias but observed an impact only on behavioural measures (willingness to approach a feared stimulus) (Najmi and Amir, 2010), and two focused on specific phobias (Reese et al, 2010; Van Bockstaele, 2011) which, according to some (e.g. MacLeod and Mathews, 2012), may be resistant to emotional change via cognitive bias modification techniques.

Two meta-analyses into the therapeutic potential of ABM have been conducted (Hakamata et al, 2010; Hallion and Ruscio, 2011). Hallion and Ruscio (2011) pooled the results of both attentional bias modification and interpretive bias modification studies. It has been suggested that the examination of the impact of bias modification was compromised by combining emotional effects measured at time points where no expectation of bias modification impacting emotion should exist (e.g. immediately after bias modification but before an emotional stressor in single session implementations) with time points where bias modification to have an emotional impact (e.g. post-treatment in multi-session implementations). The meta-analysis by Hakamata, et al. (2010) focuses exclusively on attentional bias modification, providing more relevant information on ABMT. Twelve randomised control trials with a total of 467 participants (mainly sub-clinical) were included and overall medium estimated effect sizes were reported ($d = 0.61$), with significantly larger effect sizes amongst patient populations ($d = 0.78$) when compared to non-patients ($d = 0.48$). Such clinical effect sizes begin to compare favourably with traditional psychological interventions such as CBT and/or pharmacological treatments (SSRIs) (e.g. $d = 0.86$; (Powers et al, 2008)). Thus the results of these clinical studies could be considered encouraging in terms of effectiveness of ABMT in clinical settings and suggest that ABMT has the potential to be as effective as the current first line treatments in clinical populations.

The reviewed evidence suggests that ABMT has the potential to be a useful novel psychological treatment for anxiety disorders including GAD. ABM can be 'blinded' easily (e.g. Amir et al, 2009) with majority of participants in both ABM (72%) and control (79%) conditions believing they were

not receiving an active treatment. It would be worth extending the findings from self-reports of anxiety to autonomic changes, such as a reduction in blood pressure and heart rate, and to investigate if the positive results of extinction of the attentional bias during ABMT are transferable to other techniques measuring the bias (e.g. antisaccade tasks). At this stage it is a strong candidate for both targeting the bias and resulting in a subsequent reduction in subjective anxiety.

Table 1

ABMT Studies and Outcomes.

Study	Clinical	Subclinical	Non-clinical	Delivery method	Number of ABMT sessions	Attentional bias successfully modified	Changes in anxiety	Outcomes
Studies that modified both attention and anxiety								
MacLeod, et al. (2002)			✓	Lab setting	1	✓	✓	2 groups: one trained to attend to threat and one to attend to neutral. The two groups developed differentially biased attention responses that were in accord with the assigned threat-target contingency. Both groups responded to a subsequent stress task with elevation in state anxiety; however the elevation was significantly greater in those participants who had been exposed to the attendance to threat condition as compared to attendance to neutral stimuli.

Amir, et al. (2009a)	✓SAD		Lab setting	8	✓	✓	At post-treatment, participants in the ABMT group were significantly less socially anxious and less functionally impaired than the control group. Post treatment 50% of participants in the ABMT group no longer met diagnostic criteria compared to 14% in the control condition
Amir, et al. (2009b)	✓GAD		Lab setting	8	✓	✓	A reduction in AB from pre-training to post-training in ABMT group only. 50% of participants from the ABMT group compared to 13% in control group no longer met diagnostic criteria for GAD after training.
Hayes, et al (2010)		✓ High worry	Lab setting	1	✓	✓	High worriers were assigned either to a condition requiring attention to nonthreatening words and text while ignoring worry-related material or to a mixed-attention control condition. The ABMT procedure led to fewer negative thought intrusions in a worry test than did the control condition.

Bar-Haim, et al (2011)	✓ High trait anxious	Lab setting	2	✓	✓	ABMT facilitated attention disengagement from threat, and reduced vulnerability to stress in highly anxious 10-year-olds.
Eldar, et al. (2012)	✓ Pediatric Anxiety	Clinic setting	4	✓	✓	3 conditions: ABMT + 2 control conditions: one using stimuli identical to those in the ABM condition; and one using only neutral stimuli. AB present in all groups. A reduction in AB post-training in ABMT group only. Anxiety symptoms reduced in the ABMT condition only.
Waters, et al. (2013)	✓ Pediatric Anxiety	At home	12	✓	✓	2 conditions: attention-towards-positive (searching 3 × 3 matrices for a happy face amongst angry faces); and attention-training-control (searching for a bird amongst flowers). Greater post-training attention bias towards happy faces in ATP than ATC + significantly greater reductions in clinician-rated diagnostic severity. In the ATP group, 50% of children did not meet criteria for their principal diagnosis, compared to 8% in the ATC group.

Hazen, et al. (2009)	✓ High worry	Lab setting	5	✓	✓	Either neutral-neutral, or neutral-threat word pairs. Compared to sham-training, the active retraining program produced significant reductions in both threat bias and symptoms.
Li, et al. (2008)	✓ High social anxiety	Lab setting	7	✓	✓	Attend neutral vs sham training. The training was effective in changing attentional bias in training group. Scores of the Social Interaction Anxiety Scale were reduced in training group vs sham group, while the scores of Social Phobia Scale and Negative Evaluation Scale showed no difference between the two groups.
Amir, et al. (2008)	✓ High social anxiety	Lab setting	1	✓	✓	Two conditions: attend neutral face vs control training. Those in the training condition showed significantly less attention bias to threat post training + lower levels of anxiety in response to a public-speaking challenge than did those in the control condition.

Browning, et al. (2012)	✓ History of depression		Lab setting	28	✓	✓	Positive, face-based ABM reduced both measures of recurrence risk (BDI + cortisol awakening response). This effect occurred during the month following completion of bias modification training. Word-based modification did not influence the outcome measures.
Eldar, et al. (2008)		✓	Lab setting	1	✓	✓	Those trained to attend to threat developed attentional vigilance to threat-related information. Those trained to avoid threat, had their attention unbiased. Children from both training groups reported elevated depression scores following stress-induction but only those trained to attend to threat subsequently reported elevations in anxiety.

Krebs, et al. (2010)	✓	Lab setting	1	✓	✓	Attend threat vs attend neutral words. Half of each group were given explicit instructions regarding the relationship between word valence and target location, vs half given minimal instructions. Attention had been successfully manipulated in the expected direction, + explicit instructions led to more effective attention modification. Moreover, those in the attend-threat group who received explicit instructions reported significantly more negative thought intrusions following instructed worry, vs those in the attend-neutral group.
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Hirsch, et al. (2011)	✓	Lab setting	1	✓	✓	Four conditions: (1) encouraging selective engagement with threat; (2) discouraging selective disengagement from threat; (3) discouraging selective engagement with threat; or (4) encouraging selective disengagement from threat. Those in group 1 → difference in attentional bias + a corresponding group difference in worry. This was not the case in group 4.
Dandeneau, & Baldwin (2009)	✓	Lab setting	1	✓	✓	Those in the training condition exhibited significantly less vigilance for rejection compared to those in the control condition. The attentional training also made participants with low self-esteem feel less rejected after a rejection manipulation + less willing to persevere on a virtually impossible anagrams task. Also, those in the active condition reported less interfering thoughts while completing the anagrams.

Studies that did not modify attention nor anxiety

Klumpp, & Amir (2010)	✓ Social Anxiety	Lab setting	1	*	*	3 groups: attend threat, attend neutral, control. Both Attend Threat and Attend Neutral conditions exhibited a relative decrease in anxiety in response to a speech challenge compared to those in the Control condition with no difference in anxiety between the attention training conditions.
Carlbring, et al (2012)	✓ SAD	Internet	8	*	*	No difference between groups – significant main effect of time in both groups on all anxiety measures.
Boettcher, et al. (2012)	✓ SAD	Internet	11	*	*	Significant symptom reductions in both conditions.
Boettcher, et al. (2013)	✓ SAD	Internet	14	*	*	3 groups: training attention towards positive cues; towards negative cues, and control. No AB at baseline. No change in AB post treatment in all conditions. Significant improvement in social anxiety symptoms in all 3 conditions.

Neubauer, et al (2013)	✓ SAD	Internet	8	*	*	In both groups the AB persisted at post- and follow-up assessment. Both groups significantly improved on social phobia and depression symptoms, but there were no differences between the ABMT and control groups.
Schoorl, et al (2013)	✓ PTSD	Internet	8	*	*	No AB to threat at baseline. Both ABMT and control condition led to a reduction of symptoms
Britton et al (2013)	✓ Pediatric Anxiety	Lab setting	5.7 (mean)	*	*	Anxious youth receiving 8-weeks of CBT received either ABMT training towards happy faces or placebo. Two additional comparison groups were: anxious youth receiving only CBT + healthy comparison youth. Active attention training towards happy faces did not augment clinician-rated response to CBT; however, individuals receiving training exhibited reductions on self-report measures of anxiety earlier than individuals receiving CBT only.

Rapee, et al. (2013)	✓ SAD		Home setting	39 (median)	*	*	Those receiving 12-week CBT received daily computerised training away from threat, or a placebo. At the end of treatment there were no significant differences between groups in attentional bias towards threat or in treatment response.
Kruijt, et al. (2013)		✓ High depression	Lab setting	1	*	*	Attentional training had no effect on attentional bias. Positive and negative mood states were not differentially affected by training condition.
White, et al. (2011)			✓ Lab setting	1	*	*	Although the total proportion of anxiety-related negative interpretations did not differ between training and placebo attention groups, the first interpretation generated for ambiguous scenarios was more likely to be threat-related for individuals in the training group.

AB=Attentional bias

1.5.2 Transcranial direct current stimulation (tDCS) as a candidate to target attentional deficits

Another technique which may prove to be successful in targeting attentional control deficits in anxiety is transcranial direct current stimulation (tDCS). This brain modulation technique may be used to target brain regions responsible for attentional deficits and biases directly (such as the PFC) and, potentially subsequently reduce anxiety (see section 1.1.6).

tDCS is a non-invasive brain stimulation modality, which uses weak (1-2 mA/cm²) direct current to modulate cortical brain regions. Aldini (1803) first applied a form of tDCS to patients with mood disorders and claimed to successfully improve the mood of “melancholy” patients (see Parent, 2004 for a historical overview). In the 1960s research in animals demonstrated that cortical stimulation with an anode would increase neuronal activity, while cathodal stimulation produced opposite effects (see review by Been, 2007). In contrast to transcranial magnetic stimulation (TMS) the effect of tDCS is not large enough to induce action potentials, rather tDCS alters membrane potentials to modulate the neuronal excitability (i.e. TMS stimulates; tDCS modulates).

In 1960s effects of tDCS on depression have been investigated, with mixed results. However disparities in current strength, size and positioning of the electrodes (e.g. eyebrows), lack of sham/control conditions, and unregulated administration durations (e.g. up to 8 hours a day) may have contributed to this. The first of the modern era tDCS depression studies was conducted by Boggio et al (2006) followed by Fregni et al (2008). Due to reporting large positive effects in treatment of depression using active anodal tDCS in those studies, the interest in tDCS in depression had been rekindled, which prompted Arul-Anandam and Loo (2009) to take a closer look at the mechanisms of action of this modulation technique.

1.5.2.1 Mechanisms of action

Changes in spontaneous neuronal firing rates are thought to be coupled with synaptic neuroplasticity, which contribute to intra and post tDCS effects respectively (e.g. Gonçalves & de Jesus, 2012).

It has been proposed that one non-synaptic mechanism of action is the ability to induce shifts in resting membrane potential of pre and post synaptic neurons. Cortical neurons have been found to spontaneously depolarise, even in the absence of external stimulation (e.g. Tsodyks et al, 1999) and tDCS can cause lasting changes in spontaneous neuronal activity without directly inducing action potentials (e.g. Brunoni et al, 2012). tDCS does not directly induce neuronal firing as the current densities produced in the cortex (at conventional levels of stimulation) are well below

firing thresholds (established by Tehovnik, 1996) (Wagner et al, 2007). Even though these current densities do not produce action potentials, evidence from animal studies suggests that even small voltage gradients (0.77 - 2.00 mA/cm²) can modulate neuronal firing rates. Bindman et al (1964) and Purpura and McMurtry (1965) demonstrated that anodal direct current stimulation shifts neuronal resting membrane potential towards depolarisation ('excited' neurons; increased rate of firing) and cathodal towards hyperpolarisation (inhibited neurons; reduced rate of firing) in in vivo animal studies, with 5-10 minutes of stimulation inducing effects lasting up to 5 hours (Bindman et al, 1964; Purpura & McMurtry, 1965).

Motor evoked potentials (MEP) recorded from peripheral muscles suggest that active anodal tDCS to the motor cortex (0.2-5 mA, 4s-5 minutes) increases MEP size, whereas cathodal tDCS decreases MEP size (Nitsche & Paulus, 2000). Furthermore changes in plasma calcium levels due to anodal polarisation have been observed, which can alter pH (e.g. Islam et al, 1995). An increase in pH is associated with increased neuronal activity and low pH is associated with reduced neuronal activity (Somjen & Tombaugh, 1998).

Synaptic mechanisms

Additionally, there is evidence that tDCS induces changes by altering the strength of synaptic transmission. Long Term Potentiation (LTP)⁶ and Long Term Depression (LTD)⁷ have been proposed to be induced by tDCS (e.g. Nitsche et al, 2003a). Anodal tDCS has been suggested to induce LTP through increased pre-synaptic activity coupled with post-synaptic depolarisation; and cathodal tDCS has been suggested to induce LTD through reduced pre-synaptic discharge and post-synaptic hyperpolarisation (e.g. Nitsche et al, 2003a). This view is supported by animal studies (e.g. Bindman, 1965), observing LTP and LTD after direct current polarisation in animal studies.

tDCS-induced plasticity seems to display its effects in the post administration phase (for example the blockade of NMDA⁸-glutamatergic⁹ receptors¹⁰ inhibits the effects of tDCS but does not

⁶ LTP refers to a prolonged enhancement of neurotransmission that can last hours to months, induced by concurrent activity of pre- and post-synaptic neurons (Cooke & Bliss, 2006).

⁷ LTD refers to a prolonged reduction in neuronal activity (Malenka & Bear, 2004).

⁸ N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) is an amino acid derivative that acts as a specific agonist at the NMDA receptor mimicking the action of glutamate.

⁹ Glutamic acid is the most prominent excitatory neurotransmitter, and also precursor to GABA (brain's main inhibitory neurotransmitter). Glutamate receptors are responsible for glutamate-mediated postsynaptic excitation of neural cells, and are important for neural communication, memory formation,

appear to influence the effects during tDCS administration (Nitsche et al, 2003a). Glutamate receptors, particularly for NMDA, have a role in neuronal plasticity and antidepressant response (Pittenger et al, 2007). Similarly dopaminergic mechanisms are implicated as D2 receptor¹¹ blockade (through sulpiride administration) reduces the after effects of tDCS (the tDCS-induced excitability) almost completely, suggesting that D2 receptor activation has a consolidation-enhancing effect on tDCS-induced changes of excitability in the human cortex (Nitsche et al., 2006).

Findings from imaging studies

Lang et al (2005) conducted a PET study of anodal and cathodal tDCS on the primary motor cortex. Electrodes were placed over left M1 and right frontopolar cortex, and after 10 mins (1 mA/cm²) widespread cortical and subcortical alterations in regional cerebral blood flow rCBF (increases in response to anodal and decreases in response to cathodal tDCS) were observed.

1.5.2.2 tDCS and serotonin

Serotonin is involved in learning and memory formation in animals and humans (e.g. Meneses, 1999; Ogren et al, 2008) as well as affecting LTP and LTD in slice preparations (5-HT and serotonergic receptors block or enhance LTP and LTD (e.g. Kemp & Manahan-Vaughan, 2005; Normann & Clark, 2005; Ohashi et al, 2002). Altered neuroplasticity has been proposed as a neurophysiologic condition for depression (e.g. Garcia, 2002; Popoli, Gennarelli & Racagni, 2002), with findings of stress inhibiting LTP induction but facilitating LTD generation in animals (e.g. Foy et al, 1987, Xu et al, 1997). Facilitatory plasticity can be increased by chronic SSRI administration in healthy subjects, and inhibitory plasticity can be converted into facilitation (Norman et al, 2007), suggesting that increasing serotonergic activity might accomplish its therapeutic effects by enhancing facilitatory plasticity in depression (e.g. Bearden et al, 2006).

learning and regulation. Importantly in depression memory is impaired (changes to PFC, hippocampus) and also a recall bias.

¹⁰ The NMDA receptor is a glutamate receptor and ion channel protein found in nerve cells, activated when glutamate and glycine bind to it. Once activated it allows the flow of positively charged ions through the cell membrane (Furukawa et al, 2005). The NMDA receptor is implicated in controlling synaptic plasticity and memory function (Li & Tsien, 2009).

¹¹ Dopamine receptors are implicated in many neurological processes (e.g. motivation, pleasure, memory) as well as in modulation of neuroendocrine signalling. In contrast several neuropsychiatric disorders implicate abnormal dopamine receptor signalling and/or dopaminergic nerve function (e.g. Girault & Greengard, 2004). Dopamine receptors are common neurologic drug targets e.g. most antipsychotics are dopamine receptor antagonists. D2 is encoded by dopamine receptor D2 gene, of which there are 2 forms: D2Sh (short) situated pre synaptically with modulatory functions and D2Lh (long) which is a post synaptic receptor in either excitatory or inhibitory way, unless blocked by receptor antagonist.

In a single blind, placebo controlled randomised crossover study with healthy subjects, Nitsche et al (2009) investigated the impact of serotonin on neuroplasticity in humans over 4 sessions by evaluating the impact of SSRI (citalopram) on plasticity induced by tDCS. Citalopram or placebo was administered 2 hours before the experimental session and TMS was used to elicit motor evoked potentials (MEPs) to monitor excitability changes of the motor cortical representation of the right abductor digiti minimi muscle¹². The results suggested that serotonin can modulate tDCS induced plasticity in the human motor cortex. By enhancing serotonergic activity with citalopram, anodal tDCS increased and prolonged facilitatory plasticity whereas cathodal tDCS converted the inhibitory plasticity into facilitation.

1.5.2.3 Safety

Safety protocols have been established and provide clear guidelines for administration. The accepted maximum current for human use is 2 mA. The maximum current setting (above the suggested upper limit of 2 mA) it is still within a safe range (Utz et al, 2010). There are minor risks of generating electrochemically produced toxins (e.g. Agnew, 1987). Nitsche (2008) proposed that tDCS should be performed with saline-soaked sponge electrodes in order to minimise the chemical reactions at the electrode-skin-interface. General exclusion criteria apply for tDCS studies with healthy subjects, such as: epilepsy or acute eczema under the electrodes, and metallic or electronic implants. Possible side effects may include headaches, dizziness, nausea, and an itching sensation as well as skin irritation under the electrodes (Poreisz et al, 2007). tDCS does not cause epileptic seizures nor does it reduce the seizure threshold in animals (Liebetanz et al, 2006) unlike TMS.

1.5.2.4 Comparison with other brain stimulation modalities

To date the most commonly used method of brain stimulation is TMS, which utilizes a magnetic coil held above the targeted area on the scalp, which employs rapidly changing magnetic fields to induce small electrical currents in the brain. Both tDCS and TMS can achieve an increase or decrease in neuronal activity, but the methods differ. tDCS administration has virtually no reports of pain from participants (unlike TMS), is less expensive, less difficult to sham than TMS, and more easy to apply (e.g. Monti et al, 2013). However in terms of the strength of the stimulation effect, tDCS only causes increased spontaneous cell firing, unlike TMS which causes neuron's action potentials to fire (hence tDCS is a more of a modulation rather than stimulation modality).

¹² The abductor digiti minimi (abductor minimi digiti, abductor digiti quinti, ADM) is a skeletal muscle situated on the ulnar border of the palm of the hand.

However due to the smaller effect of tDCS when compared to TMS, there is a much smaller chance of seizures (e.g. Sparing et al, 2008).

tDCS may have some advantages over TMS - tDCS may enable a clearer comparison between stimulated and non-stimulated groups, as tDCS has been found easier to conduct placebo-controlled studies with, compared to other brain stimulation modalities. With the exception of a slight itching sensation, participants rarely experience sensations related to the treatment (e.g. Gandiga et al, 2006), so for sham condition, tDCS can be delivered for several seconds and then discontinued, as the itching sensation is felt only initially (e.g. Siebner et al, 2004). It has been suggested that 'ramping' for 10 seconds at the beginning and end of tDCS, combined with a current administration duration of 30 seconds in the placebo/sham condition, can make the real tDCS (performed over 20 minutes) and placebo tDCS indistinguishable (e.g. Gandiga et al, 2006). Poreisz et al (2007) with a similar sham protocol found that only 17% of subjects could distinguish between real and sham tDCS conditions.

Such blinding may be more effective with lower current strengths (i.e. 1mA rather than 2 mA, e.g. O'Connell et al, 2012). A randomised double blind crossover trial (20 minutes of 2 mA tDCS vs sham) with tDCS-naïve healthy volunteers, deceived to think they were taking part in a tDCS memory trial over two sessions, revealed that after session one 72% of participants who received the active and 56% who received the sham tDCS correctly judged the condition (overall 65%). However in session two 89% in the active and 88% in sham condition judged correctly, suggesting not only that the blinding was not effective, but also that those naive to tDCS (no prior experience) are more likely to be blinded successfully (O'Connell et al, 2012). tDCS at 2 mA has been linked to more sensory effects than 1 mA and results in skin redness at electrode sites at active but not sham tDCS (e.g. O'Connell et al, 2012), which is why the effectiveness of blinding at 2mA may be compromised.

1.5.2.5 The use of tDCS – evidence of positive effects

A number of studies have investigated the use of tDCS in a range of conditions including Alzheimer's disease, Parkinson's disease, stroke and mood disorders (see Table 1.2 and Table 1.3 for some examples across different conditions). It has been also used in healthy humans in attempts to enhance cognitive function. To date no research has been undertaken investigating the use of tDCS in anxiety, however research into depression suggests positive effects might be predicted – both disorders are highly comorbid with one another and share similar deficits in PFC function.

Brunoni et al (2012) analysed evidence for the efficacy of active tDCS in the treatment of MDD. They identified 2 main protocols for the tDCS montage: anode on left DLPFC, cathode either right DLPFC ("bi-frontal"¹³) or left supraorbital region¹⁴ and gathered summative evidence from 3 types of investigations: preclinical trials, clinical studies and studies with healthy volunteers.

Available tDCS preclinical animal studies did not specifically evaluate experimental models of depression, but they still provide interesting insights (e.g. Takano et al, 2011; Wachter et al, 2011). In healthy rats active anodal tDCS increased brain activity (measured by fMRI) (e.g. Takano et al, 2011; Wachter et al, 2011). On the other hand the cathodal tDCS decreased blood flow (Wachter et al, 2011), which supports polarity dependent effects of tDCS.

Between 2006 and 2012 19 clinical studies with different methodologies exploring effects of tDCS on depression were published (Brunoni et al, 2012). Open label studies suggested a 32.1% improvement in symptoms in MDD (anodal tDCS administered twice a day) and a 45% improvement in symptoms in patients with bipolar, after 5 weeks (Ferruci et al, 2009b; Brunoni et al, 2011b). Randomised clinical trials reported between 58.5% to 60% improvement in symptoms in the active condition (12%-13.1% in sham), coupled with significantly larger depression score reductions on Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI) (Fregni et al, 2006; Fregni et al, 2006b). Boggio et al (2008a) reported a slightly smaller improvement in MDD (40%; after 10 sessions), but in a nonrandomized comparison the observed response to tDCS was comparable with the response to a 6-week course of fluoxetine (20 mg/day), with faster response to tDCS treatment (Rigonatti et al, 2008). A meta-analysis by Kalu et al (2012) suggested that tDCS has robust and clinically relevant effects in treating depression, reporting an effect size of 0.74 (Hedges' g) favouring active versus sham group. However, as the meta-analysis included some studies reporting discrepant findings (e.g. Palm et al, 2011; Loo et al, 2010), the weighted mean for percentage reduction of symptom severity with active tDCS was 28.9%, ranging from 14.6% (Palm et al, 2011; who allowed for concomitant antidepressant medication use) to 60% (Fregni et al, 2006a) and the percentage of patients reaching remission the weighted mean was 8.5% ranging from 0% (Loo et al, 2010; 2012; Martin et al, 2011; Palm et al, 2011) to 23.8% (Boggio et al, 2008).

Loo et al (2010) used a comparatively weaker tDCS current (state value) in sessions performed every other day (rather than daily). Moreover in a recent randomised controlled trial Loo et al (2012) found positive results in a larger trial where the treatment lasted 3 weeks (20 mins, 2mA)

¹³ Bi-frontal montage is chosen due to the prefrontal cortex asymmetry theory (see section 1.8); restoring left to right imbalance.

¹⁴ Chosen due to being a neutral site.

reporting an improvement in symptoms of 28.4% in the active and 15.9% in the sham conditions. During the trial Loo et al (2012) found tDCS to be capable to induce additional benefits, independently of mood improvement; with several participants spontaneously reported improved attention and concentration after active tDCS (under double-blind conditions).

Kalu et al (2012) concluded that active tDCS is more effective than sham in reducing depressive symptoms according to scores on standard depression scales. However, more clinically relevant outcome measures were needed, to compliment the data from depression self-report measures, such as response and remission rates (Rush et al, 2006). A more recent meta-analysis by Berlim et al (2013) of randomised double blind controlled trials of tDCS in MDD (up to year 2012; anodal tDCS of 1 mA or larger; over left DLPFC; 5 or more sessions; either as a monotherapy or augmentation strategy for MDD) focused on clinically relevant outcomes (response and remission rates) reporting 23.2% and 12.4% of participants in active and sham tDCS respectively responding to treatment with no significant difference in percentages going into remission (12.2% vs 5.4%; active and sham respectively). Importantly, most included studies permitted concurrent antidepressant use, thus the less promising results in such trials suggest that either concomitant use of certain medications may have negatively influenced the efficacy of tDCS or that these studies may suffer from floor effects (any potential for further improvement may have been limited by the fact that the subjects were already receiving treatment for MDD) (Nitsche & Paulus, 2011).

Brunoni et al (2013) investigated the difference between the effect of 2mA tDCS (30 minutes, 10 days) as a mono-therapy versus combined-treatment using the Montgomery-Asberg Depression Rating Scale (MADRS¹⁵), and found results supporting combined treatment. The combined treatment was superior to the SSRI sertraline on five and to tDCS on two items of the MADRS, whereas tDCS alone was superior to sertraline only on two items, suggesting that active treatments are superior to placebo on MADRS items, with the combined treatment appearing superior to both sertraline and to tDCS.

Treatment effects have been suggested to last up to 6 weeks post treatment (e.g. Elder & Taylor, 2014) but little is known of the duration of antidepressant effects following acute treatment. A continuation tDCS treatment for up to 6 months (3 months on a weekly basis, then 3 months once per fortnight) following a clinical response to an acute treatment course suggested a cumulative

¹⁵ MADRS measures sensitivity on 10 items: apparent sadness, reported sadness, inner tension, reduced sleep, reduced appetite, concentration difficulties, lassitude, inability to feel, pessimistic thoughts and suicidal thoughts.

probability of surviving without a relapse of 83.7% at 3 months, and 51.1% at 6 months (Martin et al, 2013). This suggests that it may be useful to incorporate top-up treatments for respondents.

To date four meta-analyses have been conducted investigating the efficacy and tolerability of tDCS in depression have been published (Kalu et al., 2012, Berlim et al., 2013 and Shiozawa et al., 2014; Meron et al, 2014). The most recent one indicated that tDCS may be efficacious for treatment of MDD, but suggested that current data do not support the use of tDCS in treatment-resistant depression, or as augmentation treatment (Meron et al, 2014).

To date there are no randomised controlled trials for the use of tDCS in anxiety. The effects of left DLPFC tDCS in depression are positive and broadly consistent, and DLPFC is the region suggested to be hypoactive in anxiety, leading to diminished attentional control. The spontaneous observations of improved attention due to active anodal left DLPFC tDCS (Loo et al, 2012) provide additional support for tDCS as a potential candidate to reduce both the attentional bias and the subsequent anxiety in clinical and subclinical anxiety.

Recently (and published after the completion of the experiments presented in this thesis) a study was published by Clarke et al (2014) that suggested tDCS of DLPFC could enhance the training of attentional bias via ABM. Healthy volunteers received either active or sham tDCS of the left DLPFC while completing either an “attend threat” or “avoid threat” ABMT task (tDCS began from the time of initiation of the attentional probe practice trials; mean current delivery duration of 17 min 13 sec). Those receiving active tDCS showed greater evidence of attentional bias acquisition in the targeted direction (toward or away from threat) compared with those in the sham condition. This study provides evidence that increasing activity in the DLPFC can enhance bias modification.

Table 2

Examples of Studies using tDCS for Potential Treatment Purposes: Area of Stimulation and Outcome

Study	Polarity*	Area	Current (mA/cm ²)	Duration	Effects
Depression ¹⁶					
Fregni et al (2006)	A/S	Left DLPFC	0.029	20 mins / 5 days	Anodal decreases depression scores (BDI)
Boggio et (2008)	A/S	Left DLPFC	0.05	20 mins/ 10 days	Anodal decreases depression scores (effects up to 30 days)
Rigonatti et al (2008)	A/S	Left DLPFC	0.057	20 mins/ 10 days	Antidepressant effects similar to 6 week course of fluoxetine (5mg/day)
Loo et al (2010)	A/S	Left DLPFC	0.029	20 mins/3d ays	Significant reductions in depressive symptoms in both A and S groups
Brunoni et al (2011)	Open label - Active	Left DLPFC	0.057	Twice a day, 20 mins/ 5 days	Significant reductions in depressive symptoms, also at 1 month follow-up
Dell'Osso et al (2011)	Open label - Active	Left DLPFC	0.057	Twice a day, 20 mins/ 5 days	Significant reductions in depressive symptoms, also at 1 week follow-up
Palm et al (2011)	A/S	Left DLPFC	0.028; later changed to 0.057	10 sessions of A + 10 sessions of S (20 mins) over 4 weeks.	No significant difference in depression scores after 2 weeks of real compared with 2 weeks of sham tDCS in patients with treatment resistant depression
Loo et al (2012)	A/S	Left DLPFC	0.057	20 mins/15 days	Significant reductions in depressive symptoms in Active group, also at 1 month follow-up

¹⁶ The depression subsection of this table includes all tDCS RCTs published to date, identified in the most recent systematic review (Meron et al, 2015).

Blumberger et al (2012)	A/S	Left DLPFC	0.057	15 sessions over 3 weeks	No significant difference in remission rates between active and sham tDCS in treatment resistant depression
Brunoni et al (2013)	A + sertraline, A + placebo pill, S + sertraline, S + placebo.	Left DLPFC	0.057	30 mins/session; 12 sessions (10 on consecutive week days + 2 at fortnightly intervals)	Active vs. sham tDCS was significantly superior for all outcomes. tDCS + placebo medication vs. sertraline + sham tDCS demonstrated comparable efficacies. Use of tDCS + placebo medication (but not sertraline + sham tDCS) was superior to placebo + sham tDCS.
Brunoni et al (2014)	A/S + Cognitive Control Therapy (CCT)	Left DLPFC	0.057	30 mins/session; 10 treatments on consecutive working days	No statistically significant differences between the groups on reductions of HDRS scores or remission rates at week 2 and at week 4. Older subjects demonstrated a stronger additional effect of tDCS when combined with CCT.
Segrave et al (2014)	A+ CCT/S+ CCT /A+placebo CCT	Left DLPFC	0.057	24 min/session; 5 sessions	At 3-week follow-up, only the A + CCT group showed significant difference from baseline MADRS scores. A significant difference in response rates at 3 week follow up (but not immediately following the 5 treatment course): A+ CCT 44%, S+ CCT 11%, A+ placebo CCT 0%.
Bennabi et al (2015)	A/S	Left DLPFC	0.057	30 mins/session; 5 consecutive days	No significant difference between A and S tDCS in the change in HDRS scores immediately upon end of treatment.
Stroke					
Boggio et al (2007)	A/C/S	M1 (hand area) of the affected (A) or unaffected (C) hemisphere	0.029	20 mins	Anodal or cathodal tDCS leads to a motor improvement
Fregni et al (2005)	A/C/S	M1	0.029	20 mins	tDCS of both the unaffected and affected hemisphere improved motor performance

Hummel et al (2006)	A/S	M1	0.04	20 mins	A tDCS improved simple motor functions (pinch force + reaction times) in chronic patients
Monti et al (2008)	A/C/S	Left fronto-temporal area	0.057	10 mins	Improvement of naming in patients with chronic non-fluent aphasia by cathodal tDCS
Parkinson's disease					
Fregni et al (2006)	A/C/S	M1 DLPFC	0.029	20 mins	A tDCS of M1 but not C or DLPFC improved motor function
Pain					
Fregni et al (2006)	A/S	M1	0.057	20 mins / 5 days	Pain improvement after A tDCS over M1 in patients with central pain due to traumatic spinal cord injury
Roizenblatt et al (2007)	A/S	Left M1 or DLPFC	0.057	20 min / 5 days	Anodal tDCS had an effect on sleep and pain that was specific to the site of stimulation: M1 treatment significantly increased sleep efficiency by 11.8%, and DLPFC stimulation significantly decreased sleep efficiency by 7.5%. Increase in sleep efficiency was associated with an improvement in fibromyalgia symptoms (as indexed by the Fibromyalgia Impact Questionnaire).
Addiction					
Boggio et al (2008)	A/C/S	Left or right DLPFC	0.057	20 mins	Both A left/C right and A right/C left decreased alcohol craving compared to a sham
Fregni et al (2008)	A/S	Left or right DLPFC	0.057	20 mins	Both left and right A tDCS decreased smoking craving compared to a sham

Note: A = Anodal, C = Cathodal, S = Sham

1.5.2.6 tDCS and cognition

A number of studies have investigated the effects of tDCS on cognition and mood. Most have targeted prefrontal regions, as impaired function in that area is associated with psychiatric disorders such as depression and schizophrenia (e.g. Drevets, 2000; Fitzgerald et al, 2006b). Some evidence exists for positive effects of tDCS on executive function (e.g. inhibitory control (anodal); planning ability (cathodal in the initial and anodal in later phases of the task; implicit probabilistic classification learning (anodal)) in healthy individuals (e.g. Hsu et al, 2011; Dockery et al, 2009; Kincses et al, 2004). Other cognitive effects have been shown, whereby anodal tDCS over either the left or right DLPFC with the cathode over the contralateral DLPFC decreased risk taking behaviour during ambiguous decision-making (Fecteau et al, 2007) and improved working memory during a sequential-letter working memory task (Fregni et al, 2005). When applied bilaterally over frontal areas during slow wave sleep anodal tDCS was found to significantly increase word-pair retention, and mood, regardless of whether the application happened during sleeping or waking (Marshall et al, 2004). Moreover, anodal tDCS of left DLPFC has been linked to an improvement in emotional state processing in healthy volunteers (in the absence of mood changes) (e.g. Nitsche et al, 2012). Collectively, these studies suggest that tDCS may be effective in improving cognitive performance.

Despite the reported positive effects of tDCS on cognition, there is controversy regarding the number of tDCS sessions needed to observe an effect. Numerous studies in healthy adult populations suggest that a single session of tDCS has the capacity to modulate cognition (especially working memory and language production). As the paradigms utilised in the reported literature, as well as the reported results differ, Horvath et al (2015) reviewed cognitive data: in order to meet inclusion criteria, the effects of tDCS on a given task had to be explored by at least two different research groups using a comparable tDCS protocol (utilising a sham condition). The review included 59 studies, and the outcome measures were pooled and subsequently divided into four categories: executive function (set-shifting, stop-signal and the Stroop task), language, memory and 'miscellaneous'. Meta-analyses did not reveal a significant effect of tDCS on any measure including neither working memory nor language production tasks.

Horvath et al (2015) suggested state dependency as a potential explanation for their findings, suggesting that the effect an external stimulus exerts on the brain (and, by extension, cognition) is influenced by the state of the brain at the time of stimulus onset (e.g. time of the day, or amount of sleep). These factors are not usually reported in the tDCS literature but are thought to vary between studies. Moreover there are some suggestions that tDCS (known to be a relatively 'weak' form of modulation) does not work in a manner which can modulate a healthy, optimally

performing brain but works only on a sub-optimally performing brain, such as in patients suffering from various conditions (e.g. Batsikadze et al, 2013). Batsikadze et al (2013) proposed that healthy subjects may have a 'ceiling effect' in a single administration protocol, which cannot be overcome with simply more intensive stimulation. However, repeated administration protocols might be candidates to enhance the efficacy of tDCS (e.g. Monte-Silva et al. 2010). Other studies validate this idea of multiple sessions, suggesting that tDCS impacts cognition via repeated exposure and, possibly, overnight consolidation (e.g. Marshall et al, 2011). These are two very interesting suggestions, which rather than disputing the effectiveness of tDCS discuss the most optimal use of tDCS in terms of number of sessions and target populations.

The Horvath et al (2015) review was criticised by Price et al (2015) as substantial methodological issues were found, including inconsistent and inappropriate data selection, mischaracterisation of examined studies, incorrect subject number, and problematic statistical analyses. Price et al (2015) identified several cases in which the outcome measure selected for the meta-analysis by Horvath et al (2015) did not represent the behaviour it stated to represent; in another case, certain experimental data sets appeared to have been left out without justification. Moreover the lack of a power analysis was criticised, especially as the majority of the analyses included only three or fewer experiments. Though there is debate over the number of studies needed for a meta-analysis, experts in the field suggest examining no fewer than six studies for a single analysis in order to provide a meaningful summary statistic (e.g. Higgins et al, 2008; Fu et al, 2011). Of 59 analyses performed by the authors however, only one in the main text includes more than five studies. In sum, Horvath's et al (2015) results questioning the efficacy of tDCS in cognition in single sessions should be viewed cautiously.

Table 3

Examples of Studies using tDCS for Examining Cognitive/Behavioural Effects: Area of Stimulation and Outcome

Study	Polarity*	Area	Current (mA/cm ²)	Duration	Effects
Learning/memory					
Antal et al (2004)	A/C	Left V5, M1	0.029	7 mins	Improved visuo-motor performance by cathodal tDCS
Antal et al (2004)	A/C	Left DLPFC	0.05	10 mins	Improved visuo-motor learning by anodal tDCS
Boggio et al (2006)	A/S	M1, left DLPFC	0.029 or 0.057	20 mins	Improvement in working memory of Parkinson's disease patients after A tDCS of the DLPFC with 2 mA but not with 1 mA
Flores et al (2008)	A/C/S	Cp5	0.029	20 mins	Enhanced language learning by anodal tDCS
Fregni et al (2006)	A/S	Left DLPFC	0.029	20 mins / 5 days	Working memory improvement after A tDCS in depressive patients
Coffman et al (2012)	Active 1mA versus Active 2mA	Inferior frontal cortex	0.029 versus 0.057	30 mins – one session	tDCS significantly affected alerting attention with larger RT difference scores for those receiving 2.0 rather than 1.0 mA.
Martin et al (2013)	A only/S only/ A during Cognitive Training/ S during CT training	Left DLPFC	0.057	30 mins / 10 days	Active tDCS+CT more accurate than sham tDCS+CT on the CT task. at follow up A tDCS+CT group but not S tDCS+CT better performance on non-trained cognitive tests (attention and working memory) Both A tDCS+CT and S tDCS+CT groups outperformed the tDCS only group at post-test.

Note: A = Anodal, C = Cathodal, S = Sham

1.6 Evaluation of novel treatments

The two novel techniques reviewed earlier (AMBT and tDCS) both have the potential to target attention bias to threat and reduce anxiety. The vast majority of studies conducted with ABMT to date include sub-clinically anxious samples; and to date no published studies exist in either sub- or clinical anxiety with tDCS. There is therefore a need to find ways to examine the efficacy of these techniques. One way of undertaking this could be using experimental models of anxiety. Experimental anxiety in humans may be induced by either chemical (e.g. CO₂ inhalation; infusion of sodium lactate) or psychological means (e.g. fear potentiated startle) (Siepmann & Joraschky, 2007). These anxiety-inducing models vary in terms of inducing acute, short-lived but intense anxiety bursts (similar to panic) (e.g. single vital capacity 35% CO₂ inhalation) and prolonged, more generalised/ trait-like anxiety symptoms (e.g. 7.5% CO₂ inhalation). Experimental models of generalised anxiety, of which the 7.5% CO₂ model is one of the most established, provide a useful way of testing the efficacy and effectiveness of novel treatments. The model temporarily mimics GAD symptoms in a healthy sample thus providing a testing stage prior to a clinical trial.

1.6.1 35% CO₂ model

The study of CO₂ inhalation in psychiatry has a long and varied history (Leake, 1973). In the late nineteenth and early twentieth century CO₂ was recognised as an anaesthetic agent (Bailey et al, 2005). The belief that these anaesthetic effects could be used to produce ‘a quietness of the central nervous system’ led to experiments in mentally disturbed patients, with findings of a temporary improvement in catatonic schizophrenia due to a 30% CO₂/70% O₂ administration (Loevenhart et al., 1929; Meduna, 1948). Moreover, substance addiction (heroin) was treated with the rapid coma technique of CO₂ inhalation therapy (70% CO₂/30% O₂) (LaVerne, 1953). Finally it was proposed that CO₂ inhalation might be used to treat maladaptive anxiety responses (e.g. specific anxiety syndromes and panic attacks) (Wolpe, 1987).

In the early 1980s the current interest in CO₂ as an experimental tool emerged, particularly to explore the neurobiology and treatment of panic disorder (Gorman et al., 1984; van den Hout and Griez, 1984). Interest in CO₂ as a model of experimental anxiety has focussed on the inhalation of either low concentrations (5–7% CO₂) over longer periods (e.g. 15–20 min) (Gorman et al., 1988), or a single vital capacity inhalation of high concentration (35%) (Verburg et al., 2001). Both models are well validated in reliably inducing panic symptoms in patients with panic disorder (Rassovsky & Kushner, 2003; Bailey et al, 2005). In healthy volunteers however, and in patients with other disorders (e.g. obsessive compulsive disorder; mood disorders) (e.g. Griez et al, 1987; Perna et al, 1995a; 1995b) the 35% CO₂ model has been shown to produce less robust and less

reliable effects in terms of panic-inducing (Bailey et al, 2005), with the exception of social phobia (e.g. Caldirola et al., 1997; Gorman et al., 1990), premenstrual dysphoric disorder (Kent et al., 2001), and bipolar disorder (Mackinnon et al., 2008) who were also found to respond to CO₂ with panic. More recently Blechert et al (2010) used 20% CO₂ inhalation with PD patients, social phobia patients and healthy controls and found that on majority of measures, including ratings of anxiety and dyspnea, social phobia patients' response profiles paralleled those of the PD patients, which is consistent with previous reports (e.g. Caldirola et al., 1997, Gorman et al., 1990, Kent et al., 2001, Wilhelm et al, 2001a; 2001b).

It is important to note that the majority of early CO₂ studies concentrated on the 35% panic-inducing dose, and used panic-specific evaluation criteria instead of a general examination of the evoked anxiety and arousal responses. Argyropoulos et al. (2002) examined the effects of a single inhalation of 35% CO₂ in normal volunteers and for the first time explored the response to CO₂ as a stress stimulus rather than as an anxiety-panic specific response. They reported a robust physiological response comprising activation of the autonomic nervous system and the HPA axis, accompanied by increases in subjective fear not amounting to panic. These findings, replicated by van Duinen et al (2005) and Kaye et al (2004) suggest that the 35% CO₂ inhalation can produce an acute stress response in healthy volunteers.

It is important to distinguish between the anxiogenic effects of the inhalation, which in the higher doses of CO₂ can induce anxiety in both healthy and clinical populations (including various anxiety and affective disorders) on a continuum scale of CO₂ sensitivity, with most robust responses in PD patients and less robust ones in healthy controls; and the ability of the inhalation to accurately mimic symptoms of anxiety in healthy subjects (i.e. acting as an experimental model).

The 35% CO₂ inhalation induces acute panic-like symptoms (more akin to extreme state anxiety), whereas the 7.5% CO₂ inhalation can be seen as a more trait-like anxiety model. The symptoms that the 7.5% induces in healthy more closely mimic chronic, generalised anxiety.

1.6.2 7.5% CO₂ model

Inhalation of air with levels of CO₂ increased to 7.5% continuously for 20 minutes can increase subjective self-report anxiety (e.g. worry, tension) as well as induce autonomic changes such as elevated autonomic arousal (e.g. heart rate, blood pressure), providing a novel experimental model of GAD in healthy humans (e.g. Bailey et al. 2005; Bailey and Nutt 2008; Bailey et al. 2011a; Poma et al. 2005). The 7.5% CO₂ model has been validated as a useful tool for evaluating existing treatments as well as novel interventions for anxiety, with evidence that anxiolytic drugs can attenuate the subjective response to the 7.5% CO₂ challenge in healthy humans (e.g. Bailey et al.

2007, 2011b). The model readily translates between animals and humans, which is helpful in better integrating the biological, behavioural and cognitive mechanisms underlying pathological anxiety and fear.

Further to the evidence of subjective and autonomic changes similar to GAD patients in the 7.5% CO₂ challenge, Garner et al (2011) demonstrated that in healthy volunteers the challenge can mimic the attentional disturbances observed in GAD. They found that inhalation of the 7.5% CO₂ for 20 minutes induced erroneous eye movements toward negative visual stimuli in an eye tracking antisaccade task. This result is consistent with evidence that patients with GAD orient more readily toward threat stimuli in other eye-tracking paradigms (e.g. Mogg et al. 2000). The evidence provides support for the 7.5% CO₂ challenge as a useful translational model which can clarify cognitive behavioural and biological mechanisms that underlie anxiety in humans, and related affective states in animals.

In animals the 7.5% CO₂ challenge suggests that inhalation of 10% CO₂ triggers a range of behaviours consistent with the anxiety phenotype in rodents, such behavioural inhibition as well as a reduced activity in the open-field test (e.g. Ziemann et al. 2009). Such behaviour reflects a common cross-species response to threat, encouraging the organism to inhibit on-going behaviour and rearrange resources to monitor and detect salient (potentially threatening) environmental stimuli. A general state of 'alertness' to sensory stimuli and facilitated orienting of attention towards detected stimuli to enable further detailed processing is a core feature of this hypervigilance. This suggests that the CO₂-induced state anxiety could contribute to changes in the orienting and alerting attentional systems (but not in the executive control), consistent with previously reviewed data in humans (Pacheco-Unguetti, 2010; 2011).

The 7.5% CO₂ model has been used with the ANT task to investigate the effects of the challenge on attention network function (Garner et al, 2012). The effects of 7.5% CO₂ vs normal air on alerting, orienting and executive control attention networks were compared and the results indicated that the CO₂ inhalation significantly improved alerting and orienting network function relative to air with executive control unaffected by inhalation condition. The results also validated previous findings, suggesting that the inhalation results in significant increases in anxiety, mood, and autonomic arousal as compared to normal air inhalation. A further analysis indicated that trait anxiety was associated with CO₂- induced increases in negative affect, heart rate, and systolic blood pressure thus suggesting that individuals with high levels of generalized trait anxiety tended to experience greater subjective and autonomic response to the CO₂ challenge. Findings by Garner et al (2011, 2012) validate the 7.5% CO₂ model of generalised anxiety suggesting that

anxiety can be rapidly and effectively experimentally induced in healthy humans, providing a useful tool to evaluate different novel treatments for anxiety, including the ABMT and tDCS.

1.7 Electroencephalogram (EEG): different pattern of activation in anxiety

Electroencephalogram (EEG) is a non-invasive method used to assess spontaneous electrical activity of the brain over a period of time and recorded from multiple electrodes placed on the scalp (e.g. Niedermeyer & da Silva, 2004). It measures voltage fluctuations resulting from ionic current within the neurons of the brain. The EEG is typically described in terms of rhythmic activity, with waveforms subdivided into bandwidths (by frequency) known as alpha, beta, theta, delta and gamma. Delta is the frequency range up to 4 Hz, with the highest amplitude and the slowest waves and in adults is usually most prominent frontally. Theta is the frequency range from 4 Hz to 7 Hz and this range has been associated with reports of relaxed, meditative, and creative states (e.g. Muir, 2010). Alpha is the frequency range from 7 Hz to 14 Hz and is seen in the posterior regions of the head on both sides, higher in amplitude on the dominant side. Beta is the frequency range from 15 Hz to about 30 Hz and is seen usually on both sides in symmetrical distribution and is most evident frontally. Gamma is the frequency range approximately 30–100 Hz and its rhythms are thought to represent binding of different populations of neurons together into a network for the purpose of carrying out a certain cognitive or motor function (e.g. Buzsaki, 2006; Elersawi, 2012).

Healthy individuals may differ from those with affective and anxiety disorders in the activation of these frequency bands (e.g. Davidson, 1998a). Davidson and Fox theorized that the frontal lobes are differentially involved in positive versus negative affective states and corresponding motivated behaviours (Davidson, 2000; Fox, 1991), with left frontal areas mediating the experience of positive emotions (e.g. joy, happiness) and approach behaviours, and right frontal areas mediating the experience of negative emotions (e.g. fear, sadness) and withdrawal behaviours, with empirical support (e.g. Davidson, 1992, 1995, 1998a). Individuals with a predisposition to depression and/or anxiety display a frontal asymmetry and exhibit differences in the alpha band activation as compared to healthy individuals - with less activation in the left frontal area in those with high anxiety and depression. Thus patterns of frontal EEG asymmetry may serve as an index of risk for a variety of emotion-related disorders, including depression and anxiety.

Recent studies have found that the pattern of resting frontal EEG alpha asymmetry is predictive of individual differences in affective style in healthy adults and children, and in some clinically

depressed and anxious populations even when their symptoms are in remission (for reviews see Davidson 1993, 2000; Davidson et al 2003; Coan and Allen 2004). Otherwise healthy adults (e.g. Sutton & Davidson 1997; Schmidt 1999) and children (for reviews see Fox 1991; Fox et al 2001) who exhibit right frontal EEG asymmetry at rest are easily distressed, fearful, and shy, whereas those who exhibit left frontal EEG asymmetry at rest are socially outgoing and extraverted. Because the pattern of frontal EEG asymmetry at rest is stable across time (e.g. Tomarken et al 1992) and context (e.g. Schmidt et al 2003) and its appearance early in life is predictive of later personality (e.g. Fox et al 2001), some have argued that this metric represents a “trait-like” marker of dispositional affective style (e.g. Davidson 1993, 2000). Studies in non-clinical samples of highly shy adults, in social anxiety, and in clinical SAD together suggest significant relative elevations in right frontal brain activity in those samples, when assessed during resting states or periods of acute emotional provocation (e.g. Schmidt, 1999; Beaton et al, 2008; Davidson et al, 2000).

Moreover in the alpha frequency band, frontal EEG asymmetry has been tentatively linked to attentional biases and proposed as a biological marker of a person’s ‘affective style’ (e.g. Davidson, 1995). Some individuals categorise stimuli that are perceived to be novel or ambiguous as threatening, triggering a withdrawal response (negative attentional bias) but for others the initial bias is marked by curiosity and an approach motivation (e.g. actively making new acquaintances). Withdrawal is linked to greater right frontal EEG activity at rest and in the face of emotional provocation (e.g. Coan & Allen, 2004a; Harmon-Jones, Gable, & Peterson, 2010); evident in healthy children and adults (e.g. Coan, Allen, & Harmon- Jones, 2001; Fox, 1991), individuals with high trait anxiety (e.g. Fox et al., 1995; Schmidt, 1999), and individuals with a current or past history of mood disorder (e.g. Allen & Kline, 2004; Kentgen et al, 2000). In contrast, greater left frontal EEG activity has been linked to approach tendencies, involving both positive emotions, such as joy (e.g. Ekman & Davidson, 1993), and negative emotions, such as anger (e.g. Harmon-Jones, 2007).

Pérez-Edgar et al (2013) investigated the cognitive mechanisms that may help elucidate the observed links between EEG asymmetry and patterns of socio-emotional functioning. Frontal EEG asymmetry patterns at rest and under social threat (a stressful speech condition) were observed among young adults. Asymmetries were, in turn, associated with performance on an emotion-face dot-probe attention bias task. Frontal EEG asymmetry at baseline did not predict attention bias patterns to angry or happy faces. However, increases in right frontal alpha asymmetry from baseline to the stressful speech condition were associated with vigilance to angry faces and avoidance of happy faces (potentially reflecting individual differences in response pattern - approach vs withdrawal during a mild stressor introduction). No similar results were found with

frontal beta asymmetry or parietal alpha asymmetry, suggesting a unique role of frontal regions, particularly the DLPFC, in cognitive control and threat detection.

Other studies found that 'neurofeedback' (biofeedback) training¹⁷ targeting specific brain areas in order to increase their activation (i.e. to counter the existing asymmetry) have shown positive effects in reducing the baseline frontal asymmetry found in anxious individuals (e.g. Garrett & Silver, 1976; Moscovitch et al; 2010). For example three studies of phobic (test) anxiety, including random assignment, alternative treatment control groups, and a wait-list control group, found that the group receiving alpha EEG enhancement training produced 33% more alpha post-treatment, with all three feedback groups demonstrating significant reductions in test anxiety, while the untreated control group and the relaxation training group experienced no significant reduction (Garrett & Silver, 1976). Moscovitch et al (2010) investigated whether improvements in symptoms due to treatment in socially anxious individuals exhibiting greater relative right frontal EEG activity at rest are associated with concomitant changes in resting brain activity. EEG activity at rest was measured in patients with SAD before and after CBT, and results indicated that patients shifted significantly from greater relative right to greater relative left resting frontal brain activity from pre- to post-treatment. Moreover, greater left frontal EEG activity at pre-treatment predicted greater reduction in social anxiety from pre- to post-treatment and lower post-treatment social anxiety (Moscovitch et al, 2010).

Recently Dadashi et al (2015) conducted a quasi-experimental study with 28 patients with GAD split into two groups: namely active group with neurofeedback treatment; and a waiting list group. Patients in both groups were evaluated at pre-test and post-test with General Anxiety Disorder Scale (GAD-7) and Global Assessment Functioning Scale (GAFs). The treatment group received fifteen 30-minute alpha training sessions and fifteen 30-minute theta brain training sessions, in the occipital area, with no intervention in the waiting list group. The results showed that increase of alpha and theta brain waves amplitude in occipital area in people with GAD was associated with increased global functioning and reduced symptoms of GAD in a treatment group, but no such change was observed in the waiting list group. The results also suggest that utilisation of neurofeedback training decreased symptoms, and occupational, social and psychological functioning level in GAD patients. Upon completion of the study a psychiatrist (double-blinded) performed the diagnostic interview again and the results indicated that the treatment group patients no longer met diagnostic criteria for GAD, but the waiting list group remained above

¹⁷ Neurofeedback is a comprehensive educational system that promotes brain growth and changes at the cellular level. Neurofeedback is a form of biofeedback that can facilitate localized changes in brain waves, as well as changes in local cerebral blood flow (rCBF). This method enhances connectivity between neurons and increases the acceptance of mental flexibility (Dadashi et al, 2015).

threshold level for diagnosis. Because the training was performed to increase both the activation of alpha and theta bands it is difficult to assign a causal effect of either band separately (although previous studies implicate the alpha band). Moreover, the choice of the occipital area also seems strange as the occipital area is not usually implicated in anxiety disorders (unlike frontal regions).

Oathes et al (2008) examined EEG gamma spectral power distributions during worry induction task in participants suffering from GAD and in healthy controls, and found the EEG gamma band useful in differentiating worry from baseline and relaxation. During worry induction, GAD patients showed higher levels of gamma activity than control participants in posterior electrode sites previously associated with negative emotion. Moreover the gamma fluctuations in these electrode sites were correlated with subjective emotional experience ratings lending additional support to interpretations of negative affect. The GAD patients subsequently underwent 14 weeks of psychotherapy followed by another gamma activity measurement during a worry task. After treatment the GAD group reported less negative affect during the worry induction task and the corresponding gamma sites that previously differentiated the clinical from control groups changed for the GAD patients in the direction of control participants. These findings provide converging evidence that GAD patients experience more negative emotion during worry and that the EEG gamma band is useful for monitoring fluctuations in pathological worry expected to follow successful treatment.

To summarise, current evidence suggests that individuals with high anxiety levels have different patterns of brain activation at rest, compared to healthy controls. In order to further validate the 7.5% CO₂ model as a reliable experimental model of generalised anxiety, EEG recording of the brain activity can be undertaken in healthy adults at rest and during the 7.5% CO₂ challenge. A similar pattern of activation in healthy volunteers undergoing the CO₂ challenge and those suffering from clinical anxiety would suggest that the model works not just in inducing physiological and subjective anxiety symptoms but also in changing brain activity.

1.8 Aims of the thesis and future directions

Novel candidates for the treatment of anxiety aim to implicitly target cognitive biases that characterise anxiety. Current evidence suggests tDCS is an effective treatment for clinical and subclinical depression. Due to the high comorbidity of anxiety and depression, and commonality in certain neuropsychological mechanisms, tDCS has been identified as a potential therapeutic intervention for anxiety. Evidence from neurocognitive models of anxiety and subsequent experimental studies suggests that deficits in activation in left DLPFC are observed in anxiety. Furthermore evidence from studies of human cognition, implicate the DLPFC in attention,

inhibition and executive control (e.g. De Raedt et al, 2010). Together this suggests that directly targeting DLPFC could reduce maladaptive attentional biases. This thesis therefore examines the effects of tDCS on subjective anxiety. Moreover, both tDCS and ABMT are investigated in their capacity to reduce selective attention to threat during an antisaccade task performed during experimentally induced anxiety.

The 7.5% CO₂ model of generalised anxiety has been used to induce a range of neuropsychological biases in attention and emotion processing characterising clinical anxiety (e.g. Garner et al, 2011) and used to evaluate both established pharmacological (SSRI paroxetine) and psychological (mindfulness) anxiety treatments. This warrants using the model to investigate whether tDCS and ABMT can result in reduction of CO₂-induced anxiety, and in altered patterns of selective attention, which would suggest their potential to modify attentional bias. Apart from using the 7.5% CO₂ model of generalised anxiety to evaluate those two novel treatments for anxiety, an EEG recording of brain activity was conducted throughout the CO₂ challenge and compared with that during normal air inhalation (at rest).

The aim of Study One (Chapter 2) was to investigate the effects of tDCS on attention network function in healthy humans using the Attentional Network Test. Specifically, left DLPFC was targeted with 20 minutes of 2 mA anodal tDCS, which was predicted to exert positive effects on executive control due to this network being anatomically linked to prefrontal regions (e.g. Fan et al, 2005). Executive control is involved in conflict resolution during competing stimuli, which evidence suggests is impaired in highly anxious individuals (e.g. Fan et al, 2005; Eysenck et al, 2007). By observing positive effects of this tDCS protocol on executive control function, the study could lend support to utilising this protocol with highly anxious populations. By collecting baseline and post-tDCS measures of state anxiety, alertness, heart-rate or blood pressure, this study was also able to inform research regarding effects of an acute tDCS administration on mood and autonomic arousal in healthy humans.

The aim of Study two (Chapter 3) was to extend previous findings and for the first time examine the effects of anodal tDCS of left DLPFC on experimentally-induced anxiety. Specifically of interest were the effects of tDCS on the response to the CO₂ challenge and a on a subsequent antisaccade task. As no other studies to date report investigations of tDCS effects on subjective anxiety, this study aim to provide the first insight into the effects of tDCS in an acute administration protocol. Moreover evidence from studies of other brain stimulation modalities and emotional attentional tasks on selective attention to threat (e.g. TMS; De Raedt et al, 2010), suggests that the stimulation of the left DLPFC results in diminished attentional engagement by threat. Guided by this, Study Two extended findings from Study One regarding the effects of tDCS on cognition and

investigated whether tDCS can improve attention control and reduce erroneous eye-movements to threat in an antisaccade task during CO₂-induced anxiety.

The aim of Study Three (Chapter 4) was to investigate the effects of the second candidate anxiety treatment - ABMT on the response to the 7.5% CO₂ challenge and the subsequent antisaccade task. Clarke et al (2014) suggest that the theoretical basis for ABMT is confirmed both by studies which successfully train attention and observe a subsequent emotional vulnerability change, and those who neither manage to train the attention nor observe an emotional vulnerability change. Study Three thus contributes to this body of research and moreover provides insight into whether attentional training prior to experimentally-induced anxiety can not only impact on hypervigilance to threat but reduce subjective and autonomic responses in an experimental CO₂ model of anxiety.

The effects of CO₂ challenge on subjective, autonomic and neuropsychological characteristics of anxiety are well known. Study Four (Chapter 5) in a within participants, extended those findings by investigating whether CO₂ challenge (vs air) induces patterns of EEG activity that characterise anxious populations, notably increased alpha in the right frontal cortex (e.g. Davidson, 1992, 1995, 1998a; Beaton et al., 2008) as well as decreased alpha in parietal and occipital regions (e.g. Budzynski & Stoyva, 1972). Research also suggests a relationship between increased EEG gamma activity and anxiety (e.g. Oya et al., 2002; Gemignani et al., 2000; Sebastiani et al., 2003), suggesting a gamma increase in left (vs right) parietal sites (Oathes et al, 2008). Recently Dadashi et al (2015) reported that increasing theta levels in occipital sites via neurofeedback in GAD patients resulted in global functioning improvements, which complements other studies reporting theta elevations being associated with meditation and relaxation (e.g. Lagopoulos et al, 2009). Consequently study Four investigates alpha, gamma and theta changes during the CO₂ challenge.

GAD is the most frequent anxiety disorder in primary care, present in 22% of patients complaining of anxiety problems (Wittchen, 2002). In 2010 it resulted in an annual estimated medical expenditures and lost productivity of between \$42.3 billion and \$46.6 billion in the United States (Ramsawh, Weisberg, Dyck, Stout, & Keller, 2011) and €74.4 billion in the EU (Olesen, Gustavsson, Svensson, Wittchen, & Jönsson, 2012). It is therefore an imperative to conduct appropriate research into potential treatment improvements. This thesis contributes to that task.

ii. Chapter 2

Effects of Transcranial Direct Current Stimulation (tDCS) on Attention Network Function.

2.1 Introduction

Clinical and sub-clinical anxiety is characterised by increased distractibility (e.g. De Raedt et al, 2010; Lapointe et al, 2013); as well as by hypervigilance to threat and negative stimuli (e.g. Mogg & Bradley, 1998). Neuropsychological models of anxiety have placed deficits in prefrontal attentional control mechanisms at the root of both the aetiology and maintenance of maladaptive ruminative thinking, worry and anxiety. For example De Raedt and Koster (2010) propose that reduced attentional control over negative stimuli (due to hypofrontality or reduced prefrontal activity) sustains negative affect. Evidence from fMRI studies indicate that activity in frontal cortical mechanisms which regulate attention in anxiety is reduced (e.g. Bishop et al, 2004, see section 1.1.6, Chapter 1).

Three fundamental attention networks have been identified through research into the functional and neuro-anatomical architecture of the attention system. These are: alerting, orienting, and executive control (Fan et al. 2002). The alerting system supports activation and maintenance of an alert state and through increasing signal-to-noise ratio; it also assists with distributed processing of temporally predicted, but not spatially localized environmental events (Aston-Jones and Cohen 2005). Orienting network is implicated in the selection and direction of resources towards the spatial location of anticipated/salient stimuli. By contrast executive control comprises higher level monitoring and conflict resolution in information processing and management of voluntary (over involuntary) responses (Botvinick et al. 2001). Orienting network has been linked to the activation in the parietal lobe and frontal eye fields (Fan et al, 2005), the alerting network to frontal and parietal regions (Coull, Nobre & Frith, 2001; Fan et al, 2005) and executive control to the ACC and lateral prefrontal cortex (Bush, Luu & Posner, 2000; Fan et al, 2005). The Attention Network Test (ANT) (see section 1.4.3., Chapter 1) is a well-established and widely used computerised task that measures these three functionally and anatomically independent networks of the human attentional system. The ANT is a simple, computerised task which is a combination of a flanker task and a cued-reaction time task. Research using the ANT has provided some evidence of deficits in executive control in participants with high trait anxiety (e.g. Pancheco-Unguetti et al, 2010). Consequently deficits in attention control are considered a possible therapeutic target in anxiety.

tDCS provides a novel therapeutic intervention that could target prefrontal deficits in attention control (see section 1.6.2., Chapter 1). In brief it is a non-invasive brain modulation method, which alters cortical tissue excitability through applying a weak constant direct current via scalp electrodes to the cortical region of interest. tDCS has been used in the treatment of a variety of disorders ranging from stroke (Fregni et al, 2005); Parkinson's disease (Boggio et al, 2006), and depression (e.g. Fregni, Boggio, Nitsche, 2006; see section 1.6.2.5., Chapter 1 for review of stimulation parameters, cortical sites and outcomes). There is evidence that left anodal tDCS of the dorsolateral prefrontal cortex (DLPFC) has beneficial effects in depression (e.g. Fregni, et al, 2006; Rigonatti, Boggio, Myczkowski, 2008), and despite no studies published on the effect of tDCS in anxiety to date, some evidence suggests that tDCS might usefully target deficits in cognition (e.g. attention control) that characterise anxiety.

2.1.1 tDCS and Cognition

A number of studies have recently investigated the effects of tDCS on cognition and mood in clinical and healthy groups. Most studies have targeted prefrontal regions in which impaired function is associated with psychiatric disorders such as depression and schizophrenia (e.g. Drevets, 2000; Fitzgerald et al., 2006b). tDCS has a positive effect on executive function in healthy individuals, for example 10 min of anodal 1.5 mA tDCS over the pre-supplementary motor area (pre-SMA¹⁸) is thought to significantly increase inhibitory control in a stop-signal task as compared to cathodal tDCS and sham (Hsu et al, 2011). There is evidence that both anodal and cathodal 1 mA tDCS of the left DLPFC for 15 minutes can improve planning ability (using the Tower of London task¹⁹), with anodal tDCS improving performance in later phases of the task, and cathodal tDCS improving the performance in initial phases of the task (Dockery et al, 2009). Other cognitive effects of prefrontal cortex stimulation have been shown, whereby 20 minutes of anodal 2 mA tDCS over either the left or right DLPFC with the cathode over the contralateral DLPFC decreased risk taking behaviour during ambiguous decision-making (using the Balloon Analog Risk Task). The task presents the participant with a balloon and offers the chance to earn money by pumping the balloon up by clicking a button, each click causing the balloon to incrementally inflate and money to be added to a counter up until a threshold, at which point the balloon is over-inflated and explodes. Each pump confers greater risk, but also greater potential reward (Fecteau et al., 2007).

¹⁸ Pre-SMA is the most anterior region of the SMA with extensive connections to the prefrontal regions (Lu, Preston, & Strick, 1994).

¹⁹ The Tower of London (TOL) is a widely used test of planning ability (Shallice, 1982), and is established as a valid measure for cognitive skill acquisition (Peretti et al., 2002). The test consists of two boards with pegs and several beads with different colours. The examiner uses the beads and the boards to present the examinee with problem-solving tasks.

Moreover, 20 minutes of 1 mA anodal tDCS of the left DLPFC has been linked to an improvement in emotional face recognition in healthy volunteers (in an absence of mood changes; Nitsche et al, 2012). This body of evidence suggests that tDCS may be effective in improving cognitive performance.

Gladwin and colleagues (2012) tested the hypothesis that anodal tDCS of DLPFC would specifically enhance the selective attention aspect of working memory by leading to information being processed in a task-relevant way when an attentional set is being imposed. (Gladwin et al, 2012, p.33). A Sternberg task with distracters was used (requiring subjects to maintain a memory set while responding to distracter stimuli). Trials started with sequential presentation of the items of a memory list followed by a delay period of 6 seconds, during which a secondary task had to be performed in two thirds of the trials, with the stimuli consisting of words drawn from the initial list, but never included in the memory set. The subjects had to respond to the colour of the words during this period, with was followed by emptying of the screen and the presentation of three probes consisting of two words from the word set, exactly one of which presented in the memory set, and the other with a 50% chance of having been used as a secondary task stimulus in the current trial (high-interference probes). Otherwise, the incorrect word had not been presented as a secondary task stimulus on that trial (low-interference probes). The goal of comparing high- and low-interference probes was to determine the ability of selective attention to keep secondary task stimuli from being confused with memory set stimuli. The results indicated that 10 minutes of 1 mA left anodal tDCS was found to enhance selective attention. When deciding which of two words had been presented in the memory set, tDCS selectively improved performance when one of those words had been presented during the delay period.

2.1.2 tDCS and Mood/Anxiety

Many studies indicate that tDCS can induce positive effects in patients with depression (see section 1.6.2.5, Chapter 1) and lead to significant mood improvements in repeated administration protocols, but relatively little research has examined the effects of prefrontal tDCS on mood and processing of emotional information in healthy individuals, and study findings are inconsistent. Several studies have found no effects of prefrontal tDCS on mood, measured by self-report (e.g. Plazier et al, 2012; Brunoni et al, 2013), whereas there have been mixed results from other studies which have used cognitive tasks to measure mood indirectly (e.g. Boggio et al, 2009; Penolazzi et al, 2010). Some reports have described improved affect: for example in an early study bilateral anodal tDCS over the frontal areas during slow wave sleep was found to significantly improve mood, regardless of whether the application happened during sleeping or waking (Marshall et al., 2004); and more recently Xu et al (2013) observed significant reduction in tension

and anxiety subscale of the Profile of Mood States (POMS) in overnight abstinent smokers following a left anodal tDCS of the DLPFC, as compared to sham.

2.1.3 Rationale and Hypotheses

Due to the limited research into the effects of tDCS on anxiety or its underlying neurocognitive mechanisms, the aim of this current study was to undertake the first examination of the effects of 20 minutes of left anodal tDCS of the DLPFC versus sham on attention network function (particularly executive control), which is known to be impaired in anxiety. Moreover, this study aimed to contribute to the limited body of literature regarding effects of anodal tDCS on mood in healthy volunteers.

The left rather than right DLPFC has been chosen as the literature suggests that brain modulation via left anodal DLPFC tDCS can lead to improvements in cognition: e.g. Boggio et al (2006) noted an improvement in working memory of Parkinson's disease patients with 2mA but not with 1mA left anodal tDCS of the DLPFC; anodal tDCS of the left DLPFC has also been linked to an improvement in emotional state processing in healthy humans (Nitsche et al, 2012); and 10 minutes of 1 mA left anodal tDCS was found to enhance selective attention in a Sternberg task (Gladwin et al, 2012). The effects of tDCS vs. sham on attention control in healthy individuals were also determined to provide an indication of whether such tDCS protocols might usefully target attentional deficits in anxiety.

Because of the positive effects of anodal tDCS of the prefrontal cortex in depression as well as on executive function in healthy humans, the hypothesis of the current study was that there will be improvements following anodal tDCS, but not sham, on the Attention Network Test, especially on the executive control network in healthy humans:

HA = anodal tDCS of the left DLPFC vs. sham tDCS in healthy humans will be associated with greater executive control on the ANT.

2.2 Method

2.2.1 Design

A between-subjects design compared two groups (active tDCS group versus control-sham tDCS) on post-tDCS measures of alerting, orienting and executive attention (ANT), and change in mood and anxiety throughout the session.

2.2.2 Participants

The study randomisation was double-blind (minimising any potential subject-expectancy as well as investigator-expectancy effects) but balanced for gender. A stratified randomisation was generated by manually producing a separate block randomisation list for males and females. Administrator blinding was achieved by having an independent researcher, not involved in data collection, program the two tDCS devices. Two different number sequences were generated and labels attached to the back of the devices – one corresponding to “condition A” and the other to “condition B”.

Thirty eligible participants were recruited and randomized to receive either 2mA active tDCS (anode over left DLPFC, cathode over right DLPFC; $n = 15$; 10 females, 5 males; mean age = 20.8 years) or non-active sham tDCS ($n = 15$; 11 females, 4 males; mean age = 21.5 years). Scalp electrodes were placed bilaterally over prefrontal sites. All participants were healthy volunteers who underwent a telephone health screen prior to the testing session. On the day of the study session participants underwent a further physical and mental health screen using a structured diagnostic interview (Mini International Neuropsychiatric Interview MINI) (Sheehan et al., 1998). This is a short structured clinical interview based on diagnostic criteria specified in DSM-IV (APA, 1994). It has been used extensively as a screening tool in healthy volunteer studies and trials (e.g. Garner, Attwood, Baldwin, James & Munafò, 2011; Garner, Attwood, Baldwin & Munafò, 2012). Eligible participants were required to be aged 18-55. Exclusion criteria included metal or electronic implants (such as pace makers, teeth implants), epilepsy, currently pregnant or breastfeeding (self-report), history of mental illness including anxiety and depression, high blood pressure ($>140/90$), use of medication in the past 8 weeks other than local treatments, paracetamol/aspirin or contraceptives, and a Body Mass Index (BMI) of < 18 , or >28 . Participants' alcohol intake was required to be no greater than 50 units per week for males or 35 units per week for females.

Recruitment and screening procedures and test protocols were approved by the Research Ethics and Governance Committee at the University of Southampton. Study Information, Consent and Debrief forms are presented in Appendices (A5-A8).

A power calculation was performed a priori for a repeated measures ANOVA with 3 variables (group x cue x congruence) which revealed that the sample size needed to detect an effect size of ($\eta^2P = 0.1$) with the alpha level of .05 and high power of 0.8 was $n=20$ (10 v 10). An effect size of at least this magnitude was expected as informed by other tDCS cognition studies (e.g. Gladwin et al (2012) found that tDCS decreased RT on the Sternberg Task ($F(1, 13) = 5.4, p = .04, \eta^2P = 0.29$)).

2.2.3 Materials

The research was conducted in labs in the Academic Unit of Psychology, Highfield Campus, Southampton.

tDCS stimulator

The HDCKit (Hand-held Direct Current) consists of a stimulator, a programmer and a set of two electrodes (Magstim, UK). The 4x4cm electrodes were encased in sponge pads soaked in a commercially available saline solution (0.83% NaCl, 0.002% EDTA, 0.00005% PHMB), manufactured by Boots Pharmacy. Two tDCS machines were programmed to administer either 20 minutes of active 2mA or sham. To help with blinding the sham stimulator was programmed to stimulate for 15 seconds (ramped from 0-2mA). Please see Appendix A1 for a detailed description of the tDCS kit. Systolic and diastolic blood pressure and heart rate were measured using an Omron arm cuff (Omron-M6, Medisave, UK).

Research Materials are presented in the Appendices (A2-A6) and detailed below.

Self-report measures (see Appendix A6 for scales)

Trait Measures

Spielberger Trait – State Anxiety Inventory (Spielberg, Gorsuch, Lushene, Vagg and Jacobs, 1983)

The State-Trait Anxiety Inventory (STAI) is a commonly used measure of trait and state anxiety. It has 20 items for assessing trait and 20 for state anxiety. State anxiety items include: “I am tense; I am worried” and “I feel calm; I feel secure.” Trait anxiety items include: “I worry too much over something that really doesn’t matter” and “I am content; I am a steady person.” All items are rated on a 4-point scale (e.g., from “Almost Never” to “Almost Always”). Higher scores indicate greater anxiety.

The STAI and SSAI have been chosen in this current research due to high internal consistency coefficients for both scales ranging from .86 to .95. The trait measures have high test-retest reliability coefficients ranging from .65 to .75 over a 2-month interval (Spielberger et al., 1983). The two questionnaires have been extensively used in both clinical and non-clinical studies of anxiety over the last twenty years.

Anxiety Sensitivity Index (Reiss, Peterson, Gursky, and McNally, 1986)

Anxiety Sensitivity Index (ASI) is an 18 item questionnaire containing 6 items for the following 3 subscales: physical concerns, cognitive concerns and social concerns. The overall stability and

utility of the hierarchical ASI factor pattern using a large sample of outpatients participating in an ongoing longitudinal study of anxiety disorders indicated good test–retest reliability and consistent patterns of inter-correlation for these factor-derived subscales across a 10-month time frame (Rodriguez et al, 2004). The measure was chosen to examine possible associations between individual differences in sensitivity to somatic cues and side-effects following the tDCS. Among clinical and non-clinical samples, the ASI has been demonstrated to have good internal consistency (range of coefficients: .79–.90) and good test–retest reliability ($r = 0.75$) (e.g. Reiss et al., 1986; Vujanovic, Arrindell, Bernstein, Norton, and Zvolensky, 2007).

Attention Control Scale (Derryberry & Reed, 2002)

The Attention Control Scale (ACS) is a self-report questionnaire that has been developed to measure individual differences in attentional control. It consists of 20 self-report items combined from a 9-item measure of attentional focusing and an 11-item measure of attentional shifting (Derryberry & Rothbart, 1988), scored on a 4-point scale (1 = almost never; 2 = sometimes; 3 = often; 4 = always) e.g. “When I am working hard on something, I still get distracted by events around me” and “When concentrating I ignore feelings of hunger or thirst”. The scale has good internal consistency ($\alpha = 0.82$ for Focusing and 0.71 for Shifting (Judah et al, 2013)).

The Penn State Worry Questionnaire (PSWQ) (Meyer et al, 1990)

The PSWQ has been widely used as a self-report measure of worry in treatment outcome studies in clinical populations (e.g. Brown et al., 1992; Borkovec & Costello, 1993; Ladouceur et al., 2000), and in non-clinical laboratory investigations (Butler et al., 1995).

PSWQ consists of 16 items; each item is rated on a scale from 1 (“not at all typical of me”) to 5 (“very typical of me”). Eleven items are worded in the direction of pathological worry, with higher numbers indicating more worry (e.g. “Once I start worrying, I cannot stop”), while the remaining five items are worded to indicate that worry is not a problem, with higher numbers indicating less worry (e.g. “I do not tend to worry about things”). Total score is calculated by summing the 11 “worry items” with the 5 reverse-scored “no worry” items, with higher PSWQ scores reflecting greater levels of pathological worry. The PSWQ demonstrates good internal consistency with a Cronbach’s alpha of 0.90 (e.g. Fresco et al, 2002).

State Measures

Visual Analogue Scale (VAS)

The visual analogue scale (VAS) is a psychometric response scale which can be used in questionnaires. It is a measurement instrument for attitudes and subjective characteristics.

Respondents specify their level of agreement with a statement by indicating with a vertical line a position along a continuous line between two end-points (e.g. “not at all” at far left and “all the time” at far right). Here six items were used: feeling 1 “alert”; 2 “worried”; 3 “happy”; 4 “relaxed”; 5 “anxious” and 6 “feel like leaving”, which were grouped into three sub-groups: positive affect (items: 3 and 4), negative affect (items: 2, 5, 6) and cognition (item 1).

The VAS can be compared to other linear scales such as the Likert scale, there is evidence that the VAS may outperform other subjective measures in terms of reproducibility and sensitivity to change in the assessment of symptoms (e.g. Grant, Aitchison, Henderson, Christie, Zare, McMurray, and Dargie (1999)).

The VAS scales have been used extensively in other research involving clinical and sub-clinical populations.

GAD7 (Kroenke, Spitzer, Williams et al, 2007)

This 7 item questionnaire is widely used to screen for generalised anxiety disorder and measure anxiety severity. The individual is asked to state how many times over the last 2 weeks, they have been bothered by any of the following problems: “Feeling nervous, anxious or on edge?”, “Not being able to stop or control worrying?”, “Worrying too much about different things?”, “Trouble relaxing?”, “Being so restless that it is hard to sit still?”, “Becoming easily annoyed or irritable?” and “Feeling afraid as if something awful might happen?”. The GAD-7 score is calculated by assigning scores of 0, 1, 2, and 3, to the response categories of “not at all,” “several days,” “more than half the days,” and “nearly every day,” respectively, and adding together the scores for the seven questions. Scores of 5, 10, and 15 are taken as the cut off points for mild, moderate, and severe anxiety, respectively.

Using the threshold score of 10, the GAD-7 has a sensitivity of 89% and a specificity of 82% for GAD (Kroenke, Spitzer, Williams et al, 2007). The measure has been extensively used in research with clinical and non-clinical populations alike.

GAD 7 was used as a state measure of anxiety to monitor any potential changes in anxiety from pre- to post tDCS. The participants were asked to state how much over the past 20 minutes, they have been bothered by any of the following problems: “Feeling nervous, anxious or on edge?”, “Not being able to stop or control worrying?”, “Worrying too much about different things?”, “Trouble relaxing?”, “Being so restless that it is hard to sit still?”, “Becoming easily annoyed or irritable?” and “Feeling afraid as if something awful might happen?”. A Visual Analogue Scale was used here, with participants asked to place a vertical line on a scale, ranging from “not at all sure” to “all of the time”.

Positive and Negative Affect Scale (Watson, Clark, & Tellegen, 1988b)

The Positive and Negative Affect Schedule (PANAS) is a brief and easy to administer tool with two 10-item mood scales (negative and positive affect). The scale has been shown to have high internal consistency (alphas of .86 and .87) and good test-retest reliability at 2-months (Watson, Clark and Tellegen, 1988). It has been widely used with using clinical and sub-clinical populations. A state version was used, asking participants to indicate how they felt in the past 20 minutes. The 10 items measuring positive affect included feeling: “enthusiastic”, “interested”, and “proud”; and the 10 items measuring negative affect included feeling: “hostile”, “jittery” and “irritable”. The answers ranged from “very slightly or not at all” to “extremely”.

Spielberger Trait – State Anxiety Inventory (Spielberg, Gorsuch, Lushene, Vagg and Jacobs, 1983)

20 items measured state anxiety, including: “I am tense; I am worried” and “I feel calm; I feel secure.” All items were rated on a 4-point scale (e.g., from “Not at All” to “Very Much So”). Participants were asked to indicate how they felt in the past 20 minutes and higher scores indicated greater anxiety.

Tasks

Attention Network Test

The ANT is a combination of a flanker task with arrows (Eriksen & Eriksen, 1974) and a cued reaction time task (Posner, 1980) - see Figure 6. Participants were instructed to classify as quickly and accurately as possible whether the central (target) arrow pointed left or right via a button press response. The target arrow was flanked by two pairs of distracter arrows. Flanker arrows either pointed in the same direction (congruent condition) or opposite direction (incongruent condition) as the target arrow. The cue types were: central, spatial, double or no cue; and the target types were either congruent or incongruent. Flanker congruence, target direction and target location was counterbalanced across spatial, double, centre and no-cue trials. Participants completed 8 randomized practice trials (2 per cue-type) followed by 128 randomized experimental trials (32 trials per cue-type condition). Stimuli were presented using Inquisit 2 Computer software, Millisecond Software, Seattle, WA.

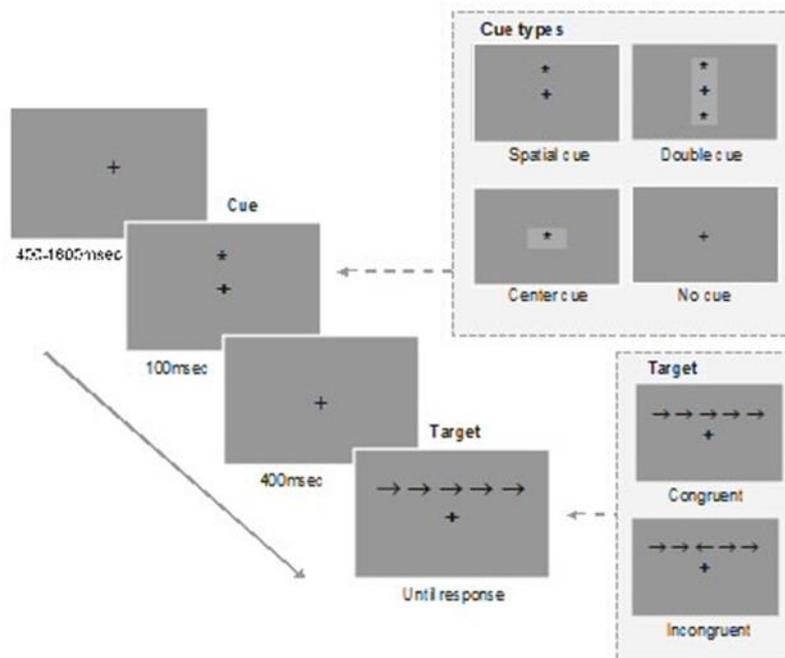


Figure 6 The Attention Network Test (adapted from Garner et al, 2011)

2.2.4 Procedure

Participants responded to a study advert and were telephone screened. Eligible participants were instructed to refrain from alcohol and excessive caffeine consumption for 24 hours prior to the test session. On the study day participants provided informed consent and underwent further screening with the MINI and measures of blood pressure/heart rate were collected. Testing was completed in a testing lab with controlled lighting and temperature. Eligible participants completed trait measures (STAI, ASI, GAD-7) and baseline state measures of anxiety and mood (SSAI, PANAS and VAS). Baseline measures of blood pressure and heart rate were taken.

tDCS

The tDCS equipment was prepared for the session (detailed description in Appendix A1). The anode was placed onto the left DLPFC area and the cathode was placed onto the right DLPFC (see Figure 7). In the active condition the machine supplied the 2mA current for 20 minutes. In the sham condition 2mA stimulation was ramped up and delivered for the first 15 seconds only. The participant was instructed to remain seated, relaxed and refrain from any motor activity for the duration of the tDCS administration.

Immediately after the 20 minutes, electrodes were removed and the following post-tDCS measures obtained: blood pressure, heart rate, and subjective ratings of peak anxiety and mood

during the tDCS period (VAS, SSAI, PANAS and GAD-7 (peak effects). Participants then completed the Attention Network Test (ANT), which lasted approximately 20 minutes.

After the completion of the initial questionnaires, the 20 minutes of tDCS, and the ANT, the participants were asked to complete a final set of supplementary questionnaires, including the ACS and PSWQ. The test session lasted approximately 2 hours. Participants were debriefed and contacted 24 hours later to discuss any further queries and register any side effects; none were reported.

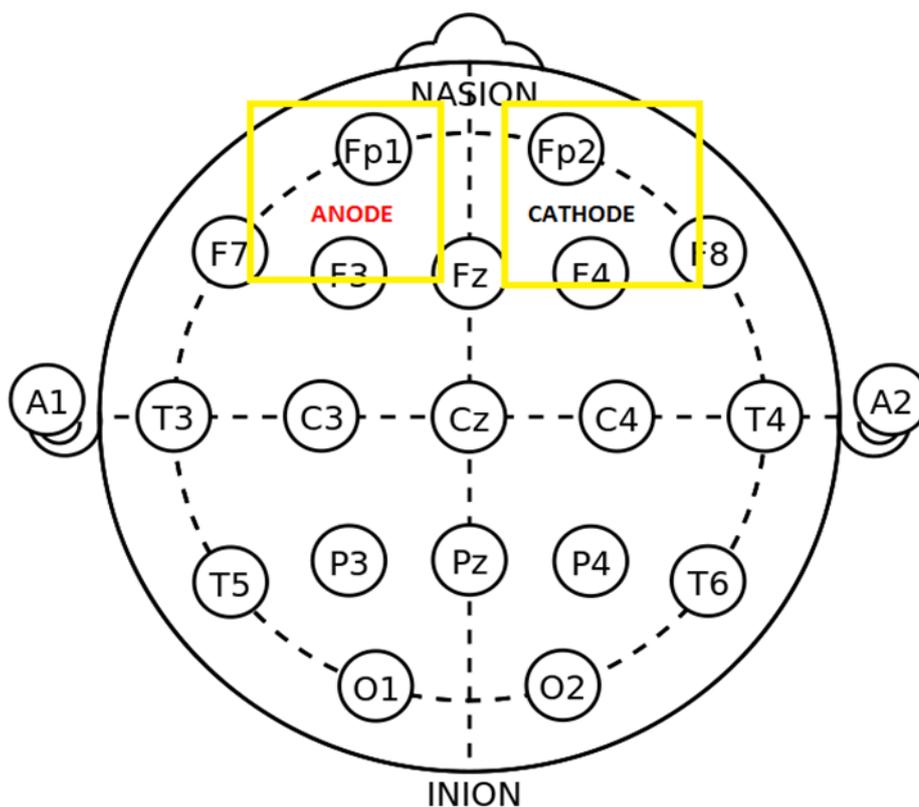


Figure 7 Bilateral tDCS Montage

2.3 Results

2.3.1 Group Characteristics

Data from 31 eligible participants were available for analysis. One participant in the sham tDCS group was an extreme outlier on task accuracy (70% accuracy) and so was removed from all analyses. Group characteristics of the remaining participants are presented in Table 4.

Independent samples t-tests indicate that active and sham tDCS groups did not significantly differ on measures of pre-existing anxiety, nor in self-report attention control. Consequently any group

differences in attention network function cannot be attributed to group differences in key pre-tDCS participant characteristics.

Table 4

Group Characteristics

	Active tDCS		Sham tDCS		t(28) =
	n=15		n=15		
	M	SD	M	SD	
GAD-7	22.07	16.55	24.19	12.84	0.39, p=.70
STAI-trait	36.13	8.46	33.73	7.00	0.85, p=.40
ACS	51.53	6.69	50.20	9.51	0.44, p=.66
ASI	32.67	9.15	32.73	9.73	-0.02, p=.99
PSWQ	47.00	10.54	45.00	26.11	0.28, p=.79
Age	20.8	1.8	21.5	2.9	0.69, p=.50
BMI	22.01	2.86	22.62	2.27	0.62, p=.54
Gender (m:f)	5:10		4:11		

ACS = Attention Control Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire

Mixed-design analyses of variance (ANOVA) tested the effects of group (active vs. sham tDCS) and time (baseline, post-tDCS) and their interaction, on anxiety, mood, heart rate and blood pressure. Principally this analysis examined i) whether tDCS produced changes in mood and arousal, and ii) whether groups differed on these measures prior to completing the ANT. Descriptive statistics for anxiety and mood measures are presented in Table 5. Descriptive statistics for autonomic arousal are presented in Table 6.

2.3.2 Anxiety and Mood

Active anodal tDCS did not modify self-report anxiety and mood measures (see Table 6). A mixed design analysis of variance (ANOVA) (2 x 2) with a between-subjects factor of group (active versus sham tDCS) and a within-subjects factor of time (baseline and post-tDCS) revealed no significant main effect of time, no significant main effect of group and no time group x interaction for GAD 7, SSAI, PANAS negative affect, and VAS positive affect (F 's (1,28) <3.41, p 's>.08).

The remaining analyses revealed significant main effects of time for PANAS positive affect, VAS negative affect and VAS cognition, indicating that following the 20 minute tDCS administration period participants (across groups) reported lower levels of positive mood ($F(1,28)=6.67$, $p=.02$, $\eta^2P=0.19$), negative mood ($F(1,28)=4.61$, $p=.04$, $\eta^2P=0.14$) and blunted cognition ($F(1,28)=26.24$, $p<.001$, $\eta^2P=0.48$). No other main effects or interactions were significant (F 's(1,28)<3.60, p 's>.07).

The results suggest that a single session of active anodal tDCS does not alter mood or subjective anxiety levels, but that participants (irrespective of group) reported less extreme mood after the 20 minute period with an improved negative affect (on VAS) but decreased positive affect (on PANAS). Possible explanations will be discussed in the Discussion section.

Table 5

Mean Measures of Self-Report Anxiety and Mood at Different Time Points.

		Active tDCS n=15		Sham tDCS n=15		t(28) =
		M	SD	M	SD	
GAD 7	Baseline	15.95	15.06	18.02	11.77	-0.42, p=.68
	Post-tDCS	14.53	14.78	14.38	13.04	0.03, p=.98
SSAI	Baseline	34.80	10.41	29.73	10.26	1.34 p=.19
	Post-tDCS	33.20	8.09	29.20	5.72	1.56, p=.13
PANAS positive	Baseline	23.73	6.23	27.80	6.21	-1.79, p=.08
	Post-tDCS	21.40	6.09	24.60	5.47	-1.51, p=.14
PANAS negative	Baseline	12.13	2.30	12.13	2.56	<0.01, p>.99
	Post-tDCS	11.33	2.00	12.80	5.93	-0.91, p=.37
VAS negative	Baseline	32.31	25.68	32.82	21.30	-0.06, p=.95

	Post-tDCS	21.70	15.46	27.36	20.85	-0.84, p=.41
VAS positive	Baseline	100.90	22.54	114.97	22.41	-1.71, p=.10
	Post-tDCS	97.93	28.67	109.10	18.90	-1.26, p=.22
VAS cognition	Baseline	94.73	20.08	106.60	27.42	-1.35, p=.19
	Post-tDCS	68.73	25.40	84.47	35.16	-1.41, p=.17

SSAI - Spielberger Trait – State Anxiety Inventory, PANAS - Positive and Negative Affect Scale; VAS - Visual Analogue Scale

2.3.3 Autonomic Arousal

Active anodal tDCS did not modify autonomic measures of systolic and diastolic blood pressure as compared to sham tDCS but both tDCS conditions had an effect on heart rate (see Table 6). ANOVA (2x2) of heart rate revealed a significant main effect of time characterised by a significant overall decrease in heart rate from pre- to post-tDCS ($F(1,28)=16.26, p <.001, \eta^2P=0.37$). There were no significant effects of group nor a significant time group x interaction ($F's(1,28)<2.34, p's>.14$).

Corresponding ANOVAs of systolic and diastolic blood pressure did not reveal any significant main effects nor interactions ($F's(1,28)<0.95, p's>.34$).

Together these results suggest that a single session of active anodal tDCS does not alter blood pressure or heart rate measures of autonomic arousal as compared to sham, but after tDCS (across groups) there were significant decreases in HR, levels of which were slightly elevated at baseline– presumably due to the stress of taking part in the experiment/lab setting.

Table 6

Heart Rate, Systolic Blood Pressure, and Diastolic Blood Pressure Mean Measures at Different Time Points.

		Active tDCS n=15		Sham tDCS n=15		t(28) =
		M	SD	M	SD	
HR	Baseline	76.67	9.45	73.27	10.67	0.92, p=.36
	Post-tDCS	68.67	11.56	69.67	9.44	-0.26, p=.80
Systolic BP	Baseline	118.87	13.86	115.60	12.27	0.66, p=.52
	Post-tDCS	117.73	22.98	113.67	11.34	0.62, p=.54
Diastolic BP	Baseline	67.33	9.85	68.93	6.93	0.02, p=.99
	Post-tDCS	71.93	23.38	66.47	8.55	0.85, p=.40

HR=Heart Rate; BP= Blood Pressure

2.3.4 tDCS and Attention Network Test

ANT scores

Consistent with previous studies and after inspection of box-plots, reaction times greater than 1000ms were removed from analysis in both groups. The alerting effect was calculated by subtracting the mean RT from double-cue trials from the mean RT on no-cue trials. The orienting effect was calculated by subtracting the mean RT on spatial-cue trials from the mean RT on centre cue trials. The executive control effect was calculated by subtracting the mean RT of all congruent trials, from the mean RT of incongruent trials. All final scores used in the analysis are thus mean reaction time difference scores (ms). Lower scores on the executive control indicate a better performance and thus higher executive control (the smaller the score, the less difference between the performance on easy congruent trials and difficult incongruent trials).

Mean Global Reaction Times and Accuracy Scores

The active and sham tDCS groups did not differ in their mean error rates (ER), ($t(28) = -1.56$, $p = .13$). Similarly the groups did not significantly differ on their mean reaction times (RT) ($t(28) =$, $p = .43$). The active anodal tDCS thus did not improve error rates nor global RTs on the ANT task (see Table 7).

Table 7

Global RTs and ERs for ANT

	Active tDCS		Sham tDCS		t(28) =
	n=15		n=15		
	M	SD	M	SD	
Mean global RT	462.10	62.57	501.94	48.56	- 0.88, $p = .43$
Mean global ER	0.04	0.04	0.02	0.02	- 1.56, $p = .13$

RT= reaction time (ms); ER= Error Rate (ER= 1-Accuracy)

Executive Control

Table 8 below shows mean reaction times for all 8 cue type x congruence conditions for each group (tDCS v Sham). The two groups did not significantly differ in their mean RTs on any of the cue-type x congruence trails.

Table 8

Mean Reaction Times in Each Cue Type x Congruence Condition in Active and Sham tDCS Groups.

	Active tDCS		Sham tDCS		t(28) =
	n=15		n=15		
	M	SD	M	SD	
Central cue congruent	472.5	101	482.6	75.4	- 0.31, $p = .76$
Central cue incongruent	557.6	84.0	573.6	62.3	- 0.59, $p = .59$
Double cue congruent	461.3	71.8	471.8	67.0	- 0.37, $p = .71$
Double cue incongruent	559.0	92.0	603.1	60.0	- 1.56, $p = .13$
Spatial cue congruent	466.1	82.8	473.7	75.0	- 0.26, $p = .79$
Spatial cue incongruent	518.6	76.0	533.4	63.4	- 0.58, $p = .57$
No cue congruent	501.5	89.6	513.3	79.4	- 0.38, $p = .71$
No cue incongruent	558.2	91.9	597.4	75.5	- 1.28, $p = .21$

An omnibus group(2) x cue-type(4) x congruence(2) mixed model analysis of variance (ANOVA) revealed a significant main effect of cue type ($F(3,28)=22.85$, $p <.001$) characterized by faster RTs on spatial cue trials ($m = 497.97$) compared to double ($m = 523.82$) and central ($m = 521.57$) cue trials, which in turn were faster than no-cue trials ($m = 542.62$), (p 's $< .01$). A significant main effect of congruency ($F(1,28)=336.94$, $p <.001$) was characterized by slower RTs on incongruent ($m = 562.62$) than on congruent trials ($m = 480.37$). This was subsumed under a significant interaction between congruency and tDCS group ($F(1,28)=4.27$, $p <.05$) – following active tDCS participants were faster on the congruent trials ($m = 475.37$) relative to incongruent trials ($m = 548.36$) compared to the sham tDCS group ($m = 485.37$, $m = 576.88$ respectively). All other results from the omnibus ANOVA were non-significant.

A series of three separate independent samples t-tests compared Active vs. Sham tDCS groups on alerting, orienting and executive control attention network scores²⁰. See Figure 8. Consistent

²⁰ Supplementary analyses examined whether group differences in alerting and orienting were moderated by target congruence (i.e. whether group differences were observed on easier congruent trials but not more challenging incongruent trials). Separate 2 (Group) x 2 (congruence) ANOVAs on alerting and orienting scores did reveal significant effects of congruency ($F(1,28)=17.28$, $p <.001$; $F(1,28)=11.71$, $p <.01$) on alerting

with the results from the omnibus ANOVA, results revealed significantly greater executive control following active than sham tDCS ($t(1,28)=2.07, p<.05$). Groups did not differ in orienting ($t(1,28)= -.19, p=.85$) nor alerting ($t(1,28)=.18, p=.86$).

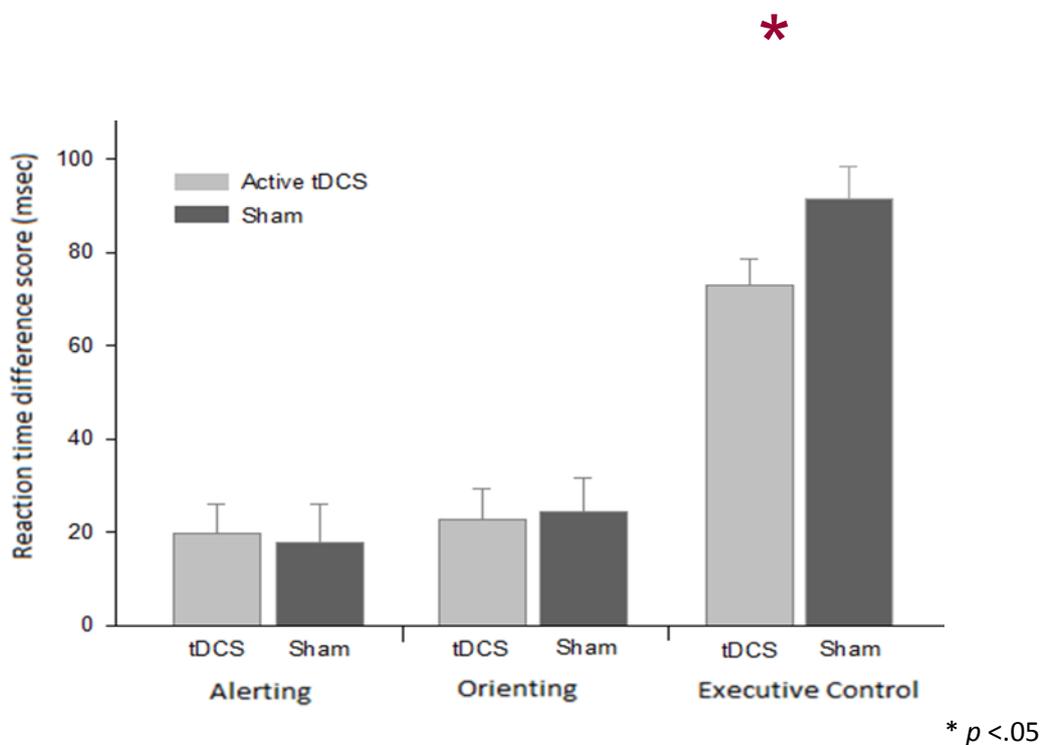


Figure 8 Mean reaction time difference between congruent and incongruent trials for ANT.

2.3.5 Blinding data

Expectancy was measured by asking participants at the end of the session to indicate their answer to the following question with a cross: “Do you think you received the active tDCS or the placebo/inactive tDCS?”. 12 participants (80%) in the active tDCS condition correctly reported receiving the active tDCS. Similarly 12 participants in the sham condition correctly reported receiving no stimulation (80%). The blinding was thus not successful at preventing participants from determining which tDCS condition they were in.

and orienting respectively, with better performance on congruent as compared to incongruent trials. There were no effects of tDCS group nor congruency x group interactions ($F's <0.01, p's >.95$) and consequently there was no evidence that tDCS affected alerting and orienting attention network function.

2.4 Discussion

In the current study executive control was significantly greater following 20 minutes of active anodal tDCS of the left DLPFC as compared to 20 minutes of sham tDCS. Both groups performed faster on the “easy” congruent trials as compared to the incongruent trials, but the difference between the incongruent and congruent trials (incongruent minus congruent) was smaller for the active anodal tDCS than for the control group.

The two groups did not differ in alerting and orienting network function. There were also no significant differences between the active and the sham groups on post-tDCS measures of anxiety, mood nor autonomic arousal.

These findings suggest that 20 minutes of active tDCS over left DLPFC is associated with greater executive control in healthy humans and support evidence from magnetic stimulation and brain imaging studies to implicate the role of the prefrontal cortex in attention control (e.g. Bishop et al, 2004).

2.4.1 Effects on cognition

The effects of tDCS on executive control complement findings from a number of recent studies in healthy volunteers, including evidence that 10 minutes of anodal 1.5 mA tDCS over the pre-supplementary motor area lead to a significantly superior inhibitory control in a stop-signal task as compared to cathodal tDCS and sham (Hsu et al, 2011). Moreover, 20 minutes of 1 mA anodal tDCS of the left DLPFC has been linked to superior emotional face recognition in healthy humans (in an absence of mood changes) (Nitsche et al, 2012). Gladwin and colleagues (2012) used a Sternberg task with distracters requiring subjects to maintain a memory set while responding to distracter stimuli and found that 10 minutes of 1 mA left anodal tDCS of DLPFC enhances selective attention. Recently Clarke et al (2014) demonstrated that sub 20 minutes of 1 mA anodal active tDCS of the left DLPFC improves attentional bias acquisition in the targeted direction (toward or away from threat) compared with participants in the sham condition. Together this body of evidence suggests that tDCS may be effective in improving attention and executive function.

At the time of devising the study presented in this chapter, no studies had been published examining the effects of tDCS on attention using the ANT. However a study has been now been published using the Attention Network Test in order to establish the effects of tDCS on attention in the context of improved learning. Coffman, Trumbo and Clark (2012) found that anodal tDCS over right inferior frontal cortex (IFC) improves learning and performance in a task where subjects learn to detect potential threats indicated by small target objects hidden in a complex virtual

environment and examined the hypothesis that these effects on learning and performance are related to changes in attention. They found that alerting, but not orienting or executive control, was significantly higher for participants receiving 2.0 mA compared with 0.1 mA tDCS thus enhancement of performance in this task may be related in part to the enhancement of alerting attention. It is important to note that in Coffman et al's (2012) study the chosen stimulated area was not DLPFC, which is the area implicated in attentional control deficits (in anxiety), and that the chosen polarity was right, not left anodal (i.e. opposite to the study presented in this chapter).

2.4.2 No effect on alerting/orienting

The current findings provide no evidence that left frontal tDCS alters alerting and orienting attention functions. Current findings differ from evidence that 2mA anodal tDCS of the right IFC for 30 minutes significantly improved alerting, but not orienting or executive attention (Coffman et al., 2012) using the Attentional Network Test (Fan et al, 2002). It is important to note that in the Coffman et al (2012) study the area of interest was not DLPFC, which is the area implicated in attentional control deficits (in anxiety), but IFC. Moreover Coffman et al's (2012) chosen polarity was right, rather than left anodal (opposite to the study described in this chapter).

Orienting network has been linked to activation in the parietal lobe and frontal eye fields (Fan et al, 2005), the alerting network to frontal and parietal regions (Coull, Nobre & Frith, 2001; Fan et al, 2005) and executive control to the ACC and LPFC (Bush, Luu & Posner, 2000; Fan et al, 2005). This suggests that stimulation of the DLPFC could be expected to have an effect on executive control; but also helps to explain perhaps the reasons for the finding reported by Coffman et al (2012) of enhanced alerting function with anodal tDCS of the frontal regions (IFC). Further investigation of the effects of tDCS on the orienting network could have implications for anxiety – patients with GAD typically display hypervigilance to threat (e.g. Bradley, Mogg, White, Groom, De Bono, 1999; Mogg, Millar, Bradley, 2000), which suggests potential deficits in the orienting network. In order to test the effect of tDCS on the orienting network the procedure would need to be revised so that the areas responsible for the orienting network could be targeted (e.g. the IFC).

The ANT is a widely used measure with high test-retest reliability (0.52 for alerting, 0.61 for orienting and 0.77 for executive control) (Fan et al, 2002). In the original report, Fan et al (2002) described the high validity of each construct, with no significant correlations between any of the attentional network scores. More recently small but significant correlations between alerting and orienting scores have been reported (Lehtonen, 2008). No significant correlations were revealed between any of the three networks in the current study (orienting and alerting $r=0.12$, $p=.95$;

orienting and executive control $r=0.30$, $p=.10$; alerting and executive control $r=0.10$, $p=.58$). Some neuroimaging evidence reveals an overlap between regions that are active during executive control and orienting, suggesting these regions might not be as functionally nor anatomically distinct as previously thought (e.g. Corbetta & Shulman, 2002).

Despite the ANT being widely used in attention research, other tasks could be considered in order to further support the finding that anodal tDCS has the capacity to improve executive control. Another widely used task is the Antisaccade Task (see Chapter 1, sections 1.1. and 1.4.2), where antisaccade errors are seen as a failure of frontally mediated inhibitory control (Clementz, 1998; Crawford, Bennett, Lekwuwa, Shaunak, & Deakin, 2002). Subsequently antisaccade errors have been linked to deficits in other cognitive constructs such as working memory, goal or intention activation, and attentional focus (Mitchell, Macrae, & Gilchrist, 2002; Nieuwenhuis, Broerse, Nielen, & de Jong, 2004; Reuter & Kathmann, 2004). The Antisaccade Task has been found to yield a reliable and sensitive measure of the processes involved in resolving the conflict between volitional and reflexive behavioural responses and is considered a reliable and comparatively simple behavioural measure that is not dependent on reaction time distributions (see Hutton & Ettinger, 2006 for a review). Consequently, it would be worthwhile to examine whether positive effects of tDCS on executive function in the ANT, extend to other measures of attention control on the antisaccade task.

2.4.3 Effects on mood

In the current study there were no acute effects of the anodal tDCS on mood or self-reported anxiety, suggesting that the observed enhancement of executive control following the active anodal tDCS occurred independently of mood. It is possible that the null effects on mood reflect the sample characteristics of predominantly young, healthy, undergraduate students. Due to the baseline mood being high for the whole sample perhaps it is not surprising that there were no further mood improvements. It is also interesting to note that post-tDCS certain mood measurements improved for both groups collectively (e.g. the magnitude of negative affect measured by VAS decreased across groups). However, despite the overall improvement in negative affect measured by VAS there was also an overall (across groups) decrease in positive affect as measured by PANAS. It should be noted that there was a trend for a baseline difference between the Active and Sham groups in terms of positive affect as measured by PANAS, characterised by higher levels of positive affect in the Sham group as compared to the Active group. Post-tDCS positive affect decreased in both groups, but the Sham group remained having a higher positive affect on average (24.60 versus 21.40 post-tDCS for sham and active groups respectively). Post-tDCS positive affect measured by VAS also decreased in both groups; and

despite this decrease not having reached statistical significance, the results of both of these affect measurements are aligned in the same direction.

These results converge with the majority of previous findings in other acute administration tDCS studies of no significant changes between the active and sham conditions (e.g. Fregni et al, 2008) where active tDCS constituted either 20 minutes of a monopolar anodal modulation of the left or right DLPFC; Nitsche et al, (2012) where active tDCS constituted 20 minutes of 1 mA anodal tDCS of the left DLPFC). An early study of bilateral anodal tDCS over the frontal areas during slow wave sleep was found to significantly improve mood, regardless of whether the application happened during sleeping or waking (Marshall et al., 2004). This was an acute administration of 2 mA anodal tDCS applied to the frontolateral locations (F3 and F4) with positive polarity at both frontal sites applied intermittently (15 sec on, 15 sec off) for 30 minutes). However, the sham condition did not apply any current and the stimulator remained off for the whole duration, which could have made the participant aware of not being in the active condition (results of any blinding procedures were not reported). Moreover the current was applied intermittently rather than constantly, which contrasts with other cited studies, in which no mood changes following single dose tDCS are reported.

Unsuccessful blinding can be problematic as it can result in demand characteristic – i.e. being aware of the type of tDCS received could lead to participants' increased motivation to perform well on a given task. In the ANT study described here, despite the unsuccessful blinding participants in the active condition did not display significant improvements in global reaction times or error rates in any of the trial types, nor any improvements in mood thus suggesting the effect of anodal tDCS on the executive control occurred independently of mood or motivation and no demand characteristics occurred.

tDCS has been reported to have positive effects in depression (e.g. Fregni et al, 2006; Boggio et al, 2008; Rigonatti et al, 2008), however these studies use tDCS as a treatment and thus administration is repeated rather than acute, with no consensus amongst the various protocols regarding the optimal number of sessions required. In the clinical studies cited above, researchers reported that anodal tDCS of the left DLPFC decreases depression scores (for up to 30 days post-tDCS) and the antidepressant effects are comparable to those of a 6 week course of fluoxetine 20mg/day. Administration of the active tDCS was for 20 minutes per session, over 5 days, 10 days and 10 days respectively.

2.4.4 Autonomic arousal

In the current study there were no acute effects of active anodal tDCS on heart rate or systolic and diastolic blood pressure suggesting that the observed enhancement of executive control following the active anodal tDCS occurred independently of changes in autonomic arousal.

Active anodal tDCS did not modify autonomic measures as compared to sham tDCS but both tDCS interventions had an effect on heart rate. A significant main effect of time characterised by a significant overall decrease in heart rate from pre to post-tDCS was revealed and in both groups the baseline measurement of HR was the highest time point measurement presumably due to the stress of being in the lab setting and the unknown element of the tDCS.

Verbal comments made by participants after tDCS session also suggest that regardless of the tDCS type received (active vs inactive) the majority of participants found the tDCS period relaxing, reflective and calming. However, it is also possible that the lowered heart rates could be attributed to acclimatisation to the laboratory setting or a relief due to the lack of pain following tDCS (which might have been a concern prior to the session).

Previous tDCS studies also report null effects of acute administrations (to other cortical sites) on blood pressure and heart rate (e.g. De Vries et al, 2010; Raimundo, Uribe & Brasil-Neto, 2012; Vandermeeren et al, 2010). De Vries et al (2010) in a between-participants study reported no effects of 20 min of 1 mA anodal tDCS over the IFC on heart rate or blood pressure in healthy volunteers as compared to sham. Similarly Vandermeeren et al (2010) reported no significant effects of 20 minutes of either anodal or cathodal mid-line frontal 1 mA tDCS on heart rate or blood pressure in healthy volunteers. The authors reported that blood pressure increased steadily in both groups, whereas the HR remained stable during a 60-minute monitoring period. Raimundo et al (2012) reported no changes in heart rate or blood pressure following 20 minutes of either the active or sham anodal tDCS (anode over the C3 position and the cathode over the right supraorbital region). Significant changes in hand skin temperature ($p < .01$) and cortisol levels ($p < .001$) after both real and sham tDCS were observed, which according to the authors were thought to reflect a non-specific stress response to a new procedure. These studies suggest that undergoing the tDCS (regardless of whether active or sham) can in itself induce some autonomic arousal changes consistent with a stress response. It should however be noted that the cited studies all utilised a weaker 1mA tDCS current strength in their protocols, and consequently they may not have reached a current strength required for a decrease in HR or perhaps there is a threshold of current strength before which tDCS leads to a stress response but after which decreases in stress response are observed.

2.4.5 Future Directions

The results of the current study indicate that active tDCS of the left DLPFC results in higher executive attention network function than did sham tDCS. Should the study be replicated, perhaps consideration should be given to a number of minor alterations. For example, additional care should be attempted in order to obtain a more even gender distribution across the two groups. The majority of the participants in this study were female, though groups did not differ in gender ratio. Research into the sex differences in cortical neuroplasticity using tDCS (e.g. Kuo, Paulus, Nitsche, 2006) has revealed some evidence that males and females respond differently to cathodal tDCS, whereby excitability-diminishing after-effects of cathodal tDCS were prolonged in females as compared to males. However, no significant differences between males and females were found for anodal tDCS.

Links with anxiety

Previous studies have utilised the ANT in populations with high anxiety and found a relationship between anxiety and impaired performance on executive control (e.g. Pacheco-Unguetti et al, 2011). Evidence suggests a distinction between the effects of trait and state anxiety on ANT function, whereby trait anxiety impairs performance on executive control and state anxiety impairs the performance on the orienting network (e.g. Pacheco-Unguetti et al, 2010). In the current study 20 minutes of active anodal tDCS of the DLPFC was associated with superior executive control. Consequently this tDCS protocol might usefully target deficits in executive control observed in trait/clinically anxious individuals.

At the time of writing this study is the first to demonstrate that 20 min 2mA tDCS of left DLPFC has a selective effect of executive attention control in healthy humans. Future studies are encouraged to examine whether anodal tDCS can improve attention control in other behavioural tasks (e.g antisaccade), and reduce hypervigilance to threat in healthy and anxious individuals.

To extend the evaluation of the effects of anodal tDCS on attentional biases present in anxiety, experimental medicine models such as 7.5% CO₂ inhalation model of generalized anxiety could be used. The model is described in detail in Chapter 1 (section 1.7.1). In brief, inhalation of air enriched with 7.5% CO₂ increases subjective self-report anxiety (e.g. worry, tension), autonomic arousal (e.g. heart rate, blood pressure) and hypervigilance to threat (Garner et al. 2011). Consequently the CO₂ experimental model of anxiety in healthy humans provides a useful tool with which to test the therapeutic benefits of tDCS for anxiety and corresponding neuropsychological deficits (Bailey et al. 2005; Bailey and Nutt 2008; Bailey et al. 2011a; Poma et al. 2005) – and this is the focus of Chapter 3.

iii. Chapter 3

Effects of Transcranial Direct Current Stimulation (tDCS) on the Subjective, Autonomic and Neuropsychological Response in the 7.5% CO₂ Experimental Model of Anxiety.

3.1 Introduction

Chapter 2 included evidence that 20 min tDCS of LPFC in healthy volunteers improves executive control (vs. sham), but does not modulate alerting and orienting network function, nor mood or autonomic arousal. This second study extends these findings to examine the effects of anodal LPFC tDCS on anxiety, autonomic arousal and attention to threat in healthy participants within an established experimental model of anxiety.

7.5% CO₂ model of generalised anxiety is described in detail in Chapter 1 (section 1.7.1). In brief, inhalation of air with levels of CO₂ increased to 7.5% can increase subjective self-report anxiety (e.g. worry, tension) as well as induce autonomic changes such as elevated autonomic arousal (e.g. heart rate, blood pressure), thereby providing a novel experimental model of GAD in healthy volunteers (Bailey et al. 2005; Bailey & Nutt 2008; Bailey et al. 2011a; Poma et al. 2005). Further to the evidence of subjective and autonomic changes similar to GAD patients in the 7.5% CO₂ challenge, recent studies suggest that in healthy individuals 7.5% CO₂ inhalation can induce a range of neuropsychological biases in attention and emotion processing that characterize clinical anxiety (review by Ainsworth and Garner, 2013). For example Garner et al (2011) demonstrated that in healthy humans the challenge can induce erroneous eye movements toward negative visual stimuli in an eye tracking antisaccade task. This result is consistent with evidence that patients with GAD orient more readily toward threat stimuli in other eye-tracking paradigms (Mogg et al. 2000) and demonstrates that CO₂ can induce hypervigilance towards and deficient inhibition of visual threat stimuli. The evidence provides support for the 7.5% CO₂ challenge as a useful translational model which can be used to evaluate the therapeutic potential of novel treatments for anxiety.

Recent studies have examined whether established, licenced pharmacological treatments for anxiety can reduce CO₂ -induced anxiety in healthy humans. A 3-week course of the selective serotonin reuptake inhibitor (SSRI) paroxetine can reduce CO₂ -induced subjective worry and

anxiety (Bailey et al., 2007). Recently Ainsworth et al (2015) used the CO₂ model to evaluate mindfulness-based interventions for generalized anxiety and found that 10 min of guided open monitoring as well as focused attention techniques completed immediately prior to inhaling 7.5% CO₂ for 20 min reduced subjective feelings of anxiety during the inhalation compared to relaxation control. These findings were consistent with neuropsychological models of mindfulness-meditation that propose open monitoring and focused attention activate prefrontal mechanisms that support emotion regulation during periods of anxiety and physiological hyperarousal. Taken together these findings suggest that 7.5% CO₂ challenge is considered a valid human experimental model of subjective, autonomic and neuropsychological features of anxiety, that can help evaluate therapeutic interventions, prior to phase-II/III clinical trials in patient populations (Bailey et al., 2011a).

In order to examine the effects of tDCS on hypervigilance to threat this study employed the Antisaccade Task (Hallett, 1978) – described in Chapter 1 (section 1.4.2). The task instructs participants to make a saccadic eye movement to the opposite side of a visual cue presented to the left or the right of the fixation point. As mentioned in section 1.1.5, anxiety impairs anti-saccade accuracy and latency i.e. with anti-saccades having longer latencies and higher error rates. Deficits in antisaccade performance have been observed in clinical samples (e.g. Jazbec et al, 2005) and in individuals with elevated levels of trait anxiety (e.g. Derakshan et al., 2009a; Ansari & Derakshan, 2011; Ansari et al., 2008). Individuals with high trait anxiety find it particularly difficult to efficiently inhibit mild-moderate threat distractors (e.g. angry facial expressions—Derakshan et al., 2009a, Exp 2; Reinholdt-Dunne et al., 2012), and show greater impairment in accuracy when processing severe threat/anxiogenic distractors (e.g. aversive images from the International Affective Picture Set; Garner et al., 2009). Moreover evidence from neuroimaging studies suggests that DLPFC activation underlies antisaccade performance (e.g. Ettinger et al, 2008).

The antisaccade task is widely used, with antisaccade errors seen as a failure of frontally mediated inhibitory control (Clementz, 1998; Crawford, Bennett, Lekwuwa, Shaunak, & Deakin, 2002). More recently antisaccade errors have also been linked to deficits in other cognitive constructs such as working memory, goal or intention activation, and attentional focus (Mitchell, Macrae, & Gilchrist, 2002; Nieuwenhuis, Broerse, Nielen, & de Jong, 2004; Reuter & Kathmann, 2004). The Antisaccade Task has been found to yield a reliable and sensitive measure of processes involved in resolving the conflict between volitional and reflexive behavioural responses and is considered a reliable and comparatively simple behavioural measure that is not dependent on reaction time distributions (see Hutton & Ettinger, 2006 for a review). Consequently, it would be worthwhile to examine whether the positive effects of tDCS on executive function in the ANT (described in

Chapter 2) extend to other measures of attention control on the antisaccade task. The antisaccade task has also been recommended for use in investigations of the effects of anxiety on attentional control (Miyake et al., 2000), following from evidence implying that anxiety impairs the functioning of the inhibition function as anxiety leads to more susceptibility to distraction (e.g. Derakshan & Eysenck, 2009).

A single study of the effects of tDCS on antisaccade performance has been published to date, and it examined whether tDCS can modulate the excitability of the frontal eye fields (FEF²¹); a key area involved in controlling eye movements and selective attention (Robinson & Fuchs, 1969; Mohler et al., 1973; Wurtz & Mohler, 1976; Schall & Thompson, 1999; Serences & Yantis, 2007). Patients with lesions to FEF and other frontal areas, including the DLPFC, have difficulty in suppressing reflexive saccades in the antisaccade task (Guitton et al., 1985) and neuroimaging studies reveal greater activation in FEF to stimuli that cue the onset antisaccade vs pro-saccade trials, consistent with the recruitment of a preparatory set for suppressing a response to the upcoming saccade target (Connolly et al., 2002; Cornelissen et al., 2002). The tDCS antisaccade study has been conducted by Kanai and colleagues (2012). Using a maximum current density of 0.11mA/cm², (which is slightly higher than typical current density ranging from 0.029 to 0.08mA/cm² (Nitsche et al., 2008), and with smaller electrode surface i.e. 3 x 3 cm rather than 4 x 4 cm) they found that bilateral tDCS of the FEF in the pro-saccade task, shortened the latency of saccades in the direction contralateral to anodal tDCS than that of saccades contralateral to cathodal tDCS. These results suggest that anodal tDCS facilitated contralateral saccade generation and/or cathodal tDCS suppressed contralateral saccade generation. There were no statistically significant effects of anodal or cathodal tDCS on accuracy rates. In the antisaccade task, the latency for ipsilateral antisaccades was prolonged during the cathodal tDCS only, whereas anodal tDCS did not modulate the latency of antisaccades. In addition, anodal tDCS reduced the erroneous saccades toward the contralateral visual cue. These results in the antisaccade task suggest that tDCS modulates the function of FEF to suppress reflexive saccades to the contralateral visual cue.

To summarise, there is emerging evidence of positive effects of anodal tDCS of the DLPFC on depressive symptoms, and on executive function in healthy volunteers (see Chapter 1, section 1.6.2.). These findings are supported by evidence included in Study One of this thesis (Chapter 2) which indicated that attentional control is greater following 20 minutes of 2 mA anodal tDCS to the left DLPFC vs sham. This second study examines whether this tDCS protocol can also improve attention control and reduce erroneous eye-movements to threat in an antisaccade task during

²¹ FEF is a region located in the frontal cortex, responsible for non-tracking, voluntary eye movements.

CO₂ -induced anxiety. The CO₂ experimental model also provides a stronger test of whether DLPFC tDCS might reduce subjective and autonomic features of anxiety induced by CO₂ - challenge.

HA = 20 minutes of active 2 mA anodal tDCS of the left DLPFC (vs sham) in healthy individuals will be followed by reduced errors and faster (correct) latencies on antisaccade trials during 7.5% CO₂ -anxiety challenge. The effect of tDCS on antisaccade performance will be greater for threat relative to neutral images.

Informed by previous literature utilising the 7.5% CO₂ model of generalized anxiety, this study predicts a robust effect of the CO₂ inhalation on subjective anxiety and autonomic arousal (relative to baseline and post tDCS). No predictions are made of a significant effect of this acute anodal tDCS administration on affect or autonomic arousal, informed by the results of Chapter 2 (section 2.4.3).

3.2 Method

3.2.1 Design

A between-subjects design compared two groups (active tDCS group versus control-sham tDCS) on subjective anxiety, autonomic arousal and antisaccade performance during 7.5% CO₂ - challenge.

3.2.2 Participants

The study randomisation was double-blind but balanced for gender. Thirty-six healthy volunteers were screened for physical and mental wellbeing and randomized to receive either 20 minute 2mA active tDCS (anode over left LPFC, cathode over right LPFC; n = 18; 12 females; mean age = 21.44 years) or non-active sham control tDCS (n = 18; 10 females; mean age = 21.29 years). The screening procedure and exclusion criteria were identical to those described in Chapter 2 (section 2.2). In brief, all participants were healthy volunteers aged 18-55, who underwent a telephone health screen prior to the testing session. On the day of the study session participants underwent a further physical and mental health screen using a pre-test diagnostic interview (Mini International Neuropsychiatric Interview MINI) (Sheehan et al., 1998).

Exclusion criteria for this study were: metal or electronic implants, epilepsy, currently pregnant or breastfeeding (self-report), history of mental health illness including anxiety and depression, high blood pressure (>140/90), use of medication in the past 8 weeks other than local treatments,

paracetamol/aspirin or contraceptives, and a Body Mass Index (BMI) of < 18, or >28. Participants' alcohol intake was required to be no greater than 50 units per week for males and 35 units per week for females.

Recruitment and screening procedures and test protocols were approved by the Research Ethics and Governance Committee at the University of Southampton. Study Information, Consent and Debrief forms are presented in Appendices (A5; A9-A10).

A power calculation was performed a priori for a repeated measures ANOVA with between-within interactions with 3 measurements (group x trial type x valence) which revealed that the sample size needed to detect an effect size of ($\eta^2P = 0.1$) with the alpha level of .05 and high power of 0.8 was $n=20$ (10 v 10). An effect size of at least this magnitude was expected, informed by the results of previous studies of antisaccade performance during 7.5% CO₂ challenge (e.g. Ainsworth et al, 2015 reports $\eta^2P = .27$ for saccade accuracy).

3.2.3 Materials

This study used the same measures as those reported in Chapter 2 (section 2.2). In brief, these included measures of trait anxiety (STAI, GAD-7, PSWQ, ASI), attention control (ACS), state mood (PANAS, SSAI, VAS) and heart rate and blood pressure. Please see Appendices (A6).

Tasks

Antisaccade Task

Eight negative and eight neutral color images were selected from the International Affective Picture Set (Center for the Study of Emotion and Motivation, Gainesville, FL, 1999). Images subtended 8×5.5 visual-deg (at 57 cm). On each trial, a word (either 'TOWARDS' or 'AWAY') was presented at central fixation for 2000 ms and instructed participants to look towards (pro-saccade) or away from (antisaccade) the picture stimulus. At 200 ms following word offset, the picture stimulus was presented for 600 ms (6° to the left or right of central fixation). On pro-saccade ('TOWARDS') trials, participants were required to look toward the picture and on antisaccade ('AWAY') trials to look away from it (ie, to shift their gaze to the opposite side of the screen). There were 96 randomized experimental trials, prior to which participants completed eight practice trials in which they completed pro- and antisaccades to a peripheral yellow rectangle. Participants additionally were asked to classify the direction of a small arrow (\uparrow or \downarrow) presented at 50 ms following picture offset (arrow-picture location congruent on 50% of trials per trial type) in order to increase task demand on each trial.

Stimuli were presented using Inquisit 2 Computer software, Millisecond Software, Seattle, WA. Horizontal eye movements were measured by electrooculography and sampled at 1000 Hz (MP150-amplifier and AcqKnowledge-3.8.1 software, Biopac-Systems, Goleta, CA).

3.2.4 Procedure

As with Chapter 2 participants responded to a study advert and were telephone screened. Eligible participants were instructed to refrain from alcohol and caffeine consumption for 24 hours prior to the test session. On the study day participants provided informed consent and underwent further screening with the MINI and blood pressure/heart rate. Testing was completed in a laboratory with controlled lighting and temperature. Eligible participants completed trait measures (STAI, ASI, GAD-7, ACS, PSWQ) and baseline state measures of anxiety and mood (SSAI, PANAS, GAD-7 state and VAS). Baseline measures of blood pressure and heart rate were taken.

tDCS

The tDCS equipment, current strength, administration duration and targeted area and montage were identical to that described in detail in Chapter 2 (section 2.2.). In brief a bilateral montage was used with the anode on the left DLPFC area and the cathode on the right DLPFC and the current strength of 2mA supplied continuously for 20 minutes in the active condition, and ramped up and delivered for the first 15 seconds only in the sham condition.

Immediately after tDCS the measures of: blood pressure, heart rate, subjective ratings of anxiety and mood were obtained. Participants were then prepared for the 7.5% CO₂ challenge, which lasted 20 minutes.

CO₂ challenge

An oral-nasal face mask (see Appendix A3) was fitted onto the participant's face, with adhesive straps securing the mask in place and ensuring a tight seal around the nose and mouth. The mask was connected via a plastic tube to an inflatable bag, which was attached to the canister containing the premixed gas mixture of air enriched with 7.5% CO₂. The canister was placed out of the participant's view and the valve was operated by the researcher, ensuring the appropriate levels of gas being maintained in the bag at all times.

Participants were asked to breathe through the mask as normal and try to refrain from excessive motor movement for the duration of the inhalation. Midway through the inhalation participants began the Antisaccade Task. Immediately following the 20 minute inhalation participants again completed state measures: GAD 7, VAS, PANAS and SSAI as well as blood pressure and heart rate

measures. Finally, participants were asked to complete a final set of supplementary questionnaires, including the Attention Control Scale (ACS) (Derryberry & Reed, 2002) and Penn State Worry Questionnaire (PSWQ) (Meyer, Miller, Metzger, Borkovec & Thomas, 1990). The test session lasted approximately 3 hours. Participants were debriefed and contacted 24 hours later to discuss any further queries and register any side effects; none were reported.

3.3 Results

3.3.1 Group Characteristics

Data from 36 participants were collected. Independent samples t-tests indicate that the active and sham tDCS groups did not significantly differ on measures of pre-existing trait anxiety, nor in self-report attention control. Consequently any group differences in antisaccade performance were not driven by group differences in trait anxiety. For group characteristics see Table 9.

Table 9

Group Characteristics at Baseline

	Active tDCS		Sham tDCS		t(34) =
	n=18		n=18		
	M	SD	M	SD	
GAD-7 trait	21.28	10.56	18.09	10.35	0.92, p=.37
STAI-trait	31.22	4.98	34.47	6.69	-1.64, p=.11
ACS	50.76	7.08	49.28	9.52	0.52, p=.61
ASI	38.00	10.32	38.94	10.72	-0.27, p=.79
PSWQ	38.22	13.44	37.06	12.05	0.27, p=.79
Age	21.44	2.87	21.29	3.16	0.15, p=.88
BMI	21.91	3.02	22.58	2.51	-0.73, p=.47
Gender (m:f)	6:12		8:10		

ACS = Attention Control Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire

3.3.2 Anxiety and mood

A mixed design analysis of variance (ANOVA) (2 x 3) with a between-subjects factor of group (active vs sham tDCS) and a within-subjects factor of time (baseline, post-tDCS and post-CO₂) revealed no main effects of group in all of the repeated self-report mood and anxiety measures (F 's < 0.94, p 's > .34) and no significant time x group interactions (F 's < 1.27, p 's > .29). Results

revealed a significant main effect of time for all but one of the repeated self-report anxiety and mood measures: GAD7 ($F(1,34)=41.06, p<.001, \eta^2P=0.55$), SSAI ($F(1,34)=87.02, p<.001, \eta^2P=0.84$), PANAS positive affect ($F(1,34)=31.39, p<.001, \eta^2P=0.50$), PANAS negative affect ($F(1,34)=28.43, p<.001, \eta^2P=0.46$), VAS negative affect ($F(1,34)=91.56, p<.001, \eta^2P=0.73$) and VAS cognition ($F(1,34)=10.25, p<.001, \eta^2P=0.23$). For VAS positive affect there was a trend in the same direction as other measures ($F(1,34)=2.57, p=.08, \eta^2P=0.07$). Paired comparisons between each time point (collapsed across group) revealed significant increases in anxiety and negative affect, and decreases in positive affect, following CO₂-challenge (relative to baseline and post-tDCS), but no differences between baseline and post-tDCS for GAD7, SSAI, PANAS negative affect, and VAS negative affect, (p 's < .05, see superscripts in Table 10). For VAS positive affect there were significant decreases in positive affect post CO₂ as compared to baseline only (decreases in positive affect post-tDCS were observed in both groups, but the post-tDCS affect was not statistically different from either the baseline or the post CO₂ affect). For PANAS positive affect there were significant differences between all three time points, with decreases in positive affect post tDCS followed by further decreases post CO₂ -challenge. Similarly there were significant differences between all three time points for VAS cognition, with alertness dropping after the tDCS but then increasing post CO₂ challenge. This suggests that a single session of active anodal tDCS does not alter mood or subjective anxiety levels, or the anxiogenic response to CO₂-challenge.

Table 10

Mean Measures of Self-Report Anxiety and Mood at Different Time Points.

		Active tDCS		Sham tDCS		t(34) =
		n=18		n=18		
		M	SD	M	SD	
GAD 7	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	28.67 ^b	8.93	0.16, p=.88
	Post-CO ₂	23.41 ^c	8.20	22.28 ^c	7.65	0.42, p=.68

PANAS negative	Baseline	12.44 ^a	4.26	12.17 ^a	2.301	0.24, p=.81
	Post-tDCS	11.78 ^a	2.76	11.50 ^a	1.98	0.35, p=.73
	Post-CO ₂	17.47 ^b	7.30	20.28 ^b	7.39	-1.13, p=.27
VAS negative	Baseline	23.20 ^a	19.63	28.65 ^a	17.56	-0.88, p=.39
	Post-tDCS	26.06 ^a	21.44	21.80 ^a	21.52	0.60, p=.56
	Post-CO ₂	75.00 ^b	20.03	79.54 ^b	19.21	0.16, p=.88
VAS positive	Baseline	67.47 ^a	14.12	69.11 ^a	13.67	-0.69, p=.49
	Post-tDCS	66.58 ^{ab}	12.59	64.64 ^{ab}	13.81	0.44, p=.66
	Post-CO ₂	63.25 ^b	29.62	56.33 ^b	18.32	0.84, p=.41
VAS cognition	Baseline	49.94 ^a	20.26	58.00 ^a	25.70	-1.04, p=.30
	Post-tDCS	46.97 ^b	21.70	49.14 ^b	30.24	-0.25, p=.81
	Post-CO ₂	65.19 ^c	36.73	71.56 ^c	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, $p < .05$

3.3.3 Autonomic arousal

Active anodal tDCS did not modify heart rate or systolic blood pressure as compared to the sham tDCS. It did however modify measures of diastolic blood pressure (see Table 11). ANOVA (2x3) of heart rate revealed a significant main effect of time ($F(1,34)=10.01$, $p<.001$, $\eta^2P=0.23$). Paired comparisons between each time point (collapsed across group) revealed significant increases in HR following CO₂ -challenge (relative to baseline and post-tDCS), but no differences between baseline and post-tDCS (p 's < .05, see superscripts in Table 11). No significant main effect of group nor a time group x interaction were observed (F 's(1,34)<1.00, p >.37).

A corresponding ANOVA of systolic blood pressure (SBP) revealed similar results, with a significant main effect of time ($F(1,34)=7.74$, $p<.001$, $\eta^2P=0.19$). Paired comparisons between each time point (collapsed across group) revealed significant increases in SBP following CO₂ -challenge (relative to baseline and post-tDCS), but no differences between baseline and post-tDCS (p 's < .05, see superscripts in Table 11). There were no significant effects of group nor a time x group interaction (F 's(1,34)<2.41, p 's >.13).

The ANOVA of diastolic blood pressure (DBP) also revealed a significant main effect of time ($F(1,34)=3.56$, $p=.03$, $\eta^2P=0.10$). Paired comparisons between each time point (collapsed across group) revealed significant decreases in DBP following the tDCS, followed by non-significant increases in DBP post CO₂ challenge (p 's < .05, see superscripts in Table 11). Upon further investigation, this post-tDCS decrease was only statistically significant in the Active group. No main effect of group and no time x group interaction were observed (F 's(1,34)< 1.51, p 's>.23).

Together these results suggest that that a single session of active anodal tDCS does not alter systolic blood pressure or heart rate measures of autonomic arousal. Interestingly a single dose of active anodal tDCS did lower diastolic blood pressure. Consistent with previous studies, inhalation of the 7.5% CO₂ mixture produced robust increases in autonomic arousal, however the effect of CO₂ on arousal was unaffected by tDCS group.

Table 11

Heart Rate, Systolic Blood Pressure, and Diastolic Blood Pressure Mean Measures at Different Time Points.

		Active tDCS		Sham tDCS		
		n=18		n=18		
		M	SD	M	SD	t(34) =
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

HR = Heart Rate; BP = Blood Pressure. Within each measure and group values with different superscripts significantly differ, $p < .05$

3.3.4 Antisaccade Task

Saccade direction and latency were scored manually and blind to trial type and inhalation condition using AcqKnowledge software. In accordance with previous literature (e.g. Garner et al, 2011) saccades with a latency less than 100 ms (i.e. anticipatory eye movements) as well as saccades which subtended less than 6 horizontal degrees (i.e. did not terminate in the location/mirror location of the stimulus) were removed from the analyses. Saccade accuracy and latencies for correct saccades were entered into separate repeated-measures analysis of variance (ANOVA) with group (Active vs Sham tDCS), trial type (pro vs antisaccade), and image valence (negative vs neutral) as independent variables.

There were 96 experimental trials (12 trials per trial type x location x valence condition). The mean correct values for accuracy from left and right visual fields were combined and the mean value was used $(x/12)^{22}$.

Accuracy

Table 12 below shows error rates (ER) for all four trial type - valence combinations split by group (tDCS v Control). A group (2) by trial type (pro- versus anti-saccade) (2) by valence (threat versus neutral) (2) ANOVA revealed a significant main effect of trial type characterised by overall (across groups) lower ERs for pro ($m = 0.04$) as compared to anti-saccade trials ($m = 0.59$) ($F(1,34)=173.71$, $p<.001$, $\eta^2P=0.84$) as well as a significant valence x group interaction ($F(1,34)=4.06$, $p=.05$, $\eta^2P=0.11$), characterised by overall (across pro- and antisaccade trials) lower error rates on negative as compared to neutrally valenced trials in the active group only ($p=.04$), with no difference between neutral and negative trials in the sham group²³. No other significant main effects or interactions emerged (F 's (2,34) < 2.55, p 's>.12).

The results from the accuracy analysis indicate that both groups were significantly more accurate on pro- as compared to anti-saccade trials. tDCS did affect saccade accuracy, with 20 minutes of

²² In order to investigate any potential effects of location (left vs right for stimuli presentation) two complementary ANOVAs (cue x valence x location x group) were run for both saccade latency and accuracy. Both ANOVAs revealed no significant main effects of location, no location x group; location x cue; location x valence; location x cue x group; location x valence x group; location x cue x valence interactions or a 4-way interaction (F 's(3,34)<1.18, p 's>.29 for latency; F 's(3,34)<2.03, p 's>.16 for accuracy).

²³ The overall valence x group interaction was investigated by running the valence analysis separately for Active and Sham groups. When running the analysis separately for pro and antisaccade trials there was a strong trend for the active group to be more accurate on the antisaccade negative as compared to antisaccade neutral trials ($F(1,17)=4.26$, $p=.06$, $\eta^2P=0.20$), which was not observed for the sham group ($F(1,17)=0.27$, $p=.61$, $\eta^2P=0.02$). There were no meaningful differences within or between groups on the pro-saccade trials.

active anodal tDCS to left DLPFC resulting in reduced selective attention to threat during experimentally induced anxiety.

Table 12

Error Rates for the Active and Control tDCS Conditions by Trial Type and Valence (score out of 12)

	Active tDCS		Sham tDCS		t(34) =
	n=18		n=18		
	M	SD	M	SD	
Pro-saccade Negative	0.03	0.06	0.04	0.04	-0.53, p=.60
Pro-saccade Neutral	0.04	0.05	0.04	0.06	0.13, p=.90
Antisaccade Negative	0.56	0.25	0.59	0.23	-0.44, p=.67
Antisaccade Neutral	0.62	0.25	0.58	0.25	0.55, p=.59

Latency

Table 13 below shows mean reaction times for all the 4 trial type - valence combinations split by group (tDCS v Control). A group (2) by cue (pro- versus anti-saccade) (2) by valence (threat versus neutral) (2) ANOVA revealed a significant main effect of cue type characterised by overall faster reaction times (RTs) for pro ($m = 168.81\text{ms}$) as compared to anti-saccade trials ($m = 276.36\text{ms}$) ($F(1,34)=235.23, p<.001, \eta^2P=0.89$) but no other significant main effects or interactions (F 's < 1.40, p 's > .25). Supplementary independent samples t-test indicate that there was a strong trend for pro-saccades towards threat to be slower following active relative to sham tDCS ($p = .06$, see table 13).

The results from the latency analysis indicate that both groups performed faster on pro- as compared to anti-saccade trials and tDCS did not modify the RTs. However there was a trend for the active group to be slower to attend to negatively valenced stimuli in pro-saccade trials.

Table 13

Mean Reaction Times for the Active and Control tDCS Conditions by Trial Type and Valence

	Active tDCS		Sham tDCS		t(34) =
	n=18		n=18		
	M	SD	M	SD	
Pro-saccade Negative	174.85	31.67	159.67	14.42	1.94, p=.06
Pro-saccade Neutral	172.78	23.26	167.94	15.26	1.08, p=.29
Antisaccade Negative	287.96	49.43	274.28	49.43	0.96, p=.35
Antisaccade Neutral	270.41	57.19	272.81	52.40	-0.12, p=.91

3.3.5 Blinding Data

Expectancy was measured by asking participants at the end of the session to indicate their answer to the following question with a cross: “Do you think you received the active tDCS or the placebo/inactive tDCS?”. 14 participants (77.8%) in the active tDCS condition correctly reported receiving active stimulation (vs. 4 (22.2%) who believed they were in the inactive condition). In the sham condition 11 participants correctly reported receiving no stimulation (61.1%) but 7 incorrectly believed they received the active tDCS (38.9%). Independent samples t-test revealed that the groups did significantly differ in their retro-expectancy ($t(1,34)=-2.50$, $p=.02$) with the sham group judging significantly less accurately whether they received active or inactive tDCS. The blinding was thus somewhat more successful than that in Study One (see section 2.3.5) at preventing participants from determining which condition they were in; however only in the sham group. This suggests that the 2 mA active tDCS modulation results in highly accurate retro-expectancy in the active group, with a moderate success at blinding in the sham group. The fact that the blinding was moderately successful in the sham group minimises the chances of subject-expectancy effects.

3.4 Discussion

This second study examined whether 20 minutes of left anodal tDCS of DLPFC can improve attention control and reduce erroneous eye-movements to threat in an antisaccade task during CO₂-induced anxiety. This was a novel study, as it investigated not only changes in mood due to the tDCS, but also provided a stronger test of whether tDCS of DLPFC might reduce subjective and autonomic features of anxiety induced by the CO₂ experimental model. Moreover, to date no tDCS studies have examined hypervigilance to threat using an antisaccade task.

The results indicate that 20 minutes of active 2 mA anodal tDCS of the left DLPFC (vs. sham) in healthy individuals resulted in significantly lower error rates on threat as compared to neutrally valenced trials (across pro- and antisaccade trials). No such difference was observed in the sham group. The experimental hypothesis predicted that the active tDCS group would exhibit a superior (more accurate) performance on the antisaccade as compared to pro-saccade trials during 7.5% CO₂-anxiety challenge, and that the effect of tDCS on antisaccade performance will be greater for threat relative to neutral images. This trend was observed but did not reach statistical significance ($p=.06$). Another prediction was that the active anodal tDCS would result in faster (correct) latencies on the antisaccade trials. This was not observed in the current study. There was however a strong trend for the active group to be slower on the pro-saccade trials towards threat as compared to the sham group ($p = .06$).

Informed by previous literature utilising the 7.5% CO₂ model of generalized anxiety, this study predicted a robust effect of CO₂ inhalation on subjective anxiety and autonomic arousal (relative to baseline and post tDCS), and this was observed. No predictions were made of a significant effect of this acute anodal tDCS administration on mood, and none were observed, thus the lack of changes in mood in the acute tDCS administration suggests that the effects of tDCS on the attentional bias in the active group occurred independently of current mood. This study adds to the body of evidence on the effects of tDCS on mood, and extends previous findings by examining the effects of tDCS on anxiety following an established anxiety inducing challenge (7.5% CO₂ model). Consequently evidence from this study (Study Two) as well as that from Study One (Chapter 2) suggests that this tDCS protocol affects neither mood nor anxiety in unchallenged individuals, but also not in those experiencing elevated levels of experimentally induced anxiety. Thus the evidence from this study further suggests that the effect of 20 minutes of left anodal tDCS of DLPFC predominantly affects cognitive aspects of anxiety rather than subjective or autonomic anxiety levels.

3.4.1 Effects of tDCS on Mood and Subjective Anxiety

The current study revealed that a single session of active anodal tDCS of the left DLPFC did not produce significant changes in terms of mood and subjective anxiety levels. In both groups, significant increases in subjective anxiety (GAD7, SSAI) and negative affect (PANAS, VAS) and decreases in positive affect (PANAS, VAS), following the CO₂ -challenge were observed, as predicted. The only measure, for which a significant change post-tDCS occurred (across both active and sham groups) was PANAS positive affect, where significant decreases in positive affect post tDCS were followed by further decreases post CO₂ -challenge. There were no differences however between the active and sham group, thus collectively these results suggest that a single session of active anodal tDCS does not alter mood or subjective anxiety levels, or the anxiogenic response to CO₂ -challenge.

This finding is consistent with majority of other studies that do not reveal effects of acute administration of tDCS on mood in healthy volunteers (e.g. Fregni et al, 2008; Plazier et al, 2012; Brunoni et al, 2013). In a crossover study (Fregni et al 2008) where healthy volunteers received three different types of 2 mA 20 minutes bilateral tDCS of DLPFC in 48 hour intervals (anode left/cathode right; anode right/cathode left; and sham tDCS) there were no reported effects of tDCS on mood. Plazier et al (2012) confirmed the lack of significant mood changes in a sample of 17 healthy subjects during 20 minutes of bifrontal (anodal and cathodal placement on right and left DLPFC) or bioccipital 1.5 mA tDCS, using a placebo-controlled design. More recently Brunoni et al (2013) in a within-subject design using a bifrontal tDCS montage (left anodal/right cathodal

or left cathodal/right anodal) of DLPFC, or sham, and using neutral or negatively valenced images during each tDCS session, reported that 30 minutes of anodal tDCS of left DLPFC revealed no specific changes of subjective mood and anxiety post-tDCS (using VAS).

A number of studies using tDCS either as a tool for the treatment of depression or for exploring cognitive function in healthy volunteers have reported changes in mood and anxiety self-report measures following repeated administration. For example in patients with depression, Palm et al (2012) applied either 1 or 2 mA anodal tDCS for 20 minutes/day for 2 weeks (cross-over design), over the DLPFC and found a trend for improved positive affect (PANAS).

Apart from potentially being due to acute rather than repeat administration, the lack of significant post-tDCS changes in mood between active and sham groups could reflect the predominantly high levels of mood in this young healthy volunteer sample. As baseline mood was high for the whole sample further mood improvements may have been difficult to achieve.

For both groups anxiety levels and negative mood did increase during the 7.5% CO₂ inhalation and remained elevated post-inhalation, further supporting this experimental model of GAD.

3.4.2 Effects on Autonomic Arousal

Consistent with previous studies, inhalation of the 7.5% CO₂ mixture produced robust increases in autonomic arousal characterised by significant increases in HR and SBP following CO₂-challenge, further validating the experimental model. The effect of CO₂ on HR and blood pressure was unaffected by tDCS group.

A single session of active anodal tDCS did not alter systolic blood pressure or heart rate measures of autonomic arousal, which is consistent with other tDCS studies, which report null effects on blood pressure and heart rate in healthy volunteers (e.g. Baker, Rodern & Fridriksson, 2010; Raimundo, Uribe & Brasil-Neto, 2012). Interestingly a single dose of active anodal tDCS did lower diastolic blood pressure. Significant decreases in DBP were observed following tDCS, followed by non-significant increases in DBP post CO₂ challenge. Upon further investigation, this post-tDCS decrease was only statistically significant in the Active group. This is an interesting finding, as majority of the reviewed literature suggests no autonomic arousal changes due to an acute tDCS administration.

Baker et al (2010) reported no difference in HR, SBP and DBP between active anodal and sham tDCS (1 mA, 20 minutes over the left frontal cortex with the reference electrode on the shoulder). Similarly, Raimundo et al (2012) reported no changes in heart rate or blood pressure following 20 minutes of either the active or sham anodal tDCS (anode over the C3 position and the cathode

over the right supraorbital region). However, after both real and sham tDCS the authors observed significant changes in skin temperature and cortisol levels which they attributed to a non-specific stress response to a new procedure. In contrast, all autonomic arousal measures in the study described in this chapter, decreased post-tDCS in both groups, with the decrease in DBP post active tDCS being statistically significant as compared to the baseline, suggesting an overall calming rather than stress-inducing effect of the 20 minutes tDCS session.

3.4.3 Effects on Task Performance

Accuracy

As expected, both groups displayed lower ERs for pro- as compared to anti-saccade trials. 20 minutes of active anodal tDCS of the left DLPFC resulted in overall significantly lower error rates on threat as compared to neutrally valenced trials (across pro- and antisaccade trials). No such difference between neutral and threat trials was observed in the sham group. Supplementary analyses revealed a strong trend for the active group to have superior accuracy levels on the antisaccade threat trials as compared to antisaccade neutral trials – a trend not observed for the sham group. There were no meaningful differences within or between groups on the pro-saccade trials. Collectively the results from the accuracy analysis indicate that active anodal tDCS did modify saccade accuracy, with 20 minutes of active anodal tDCS to left DLPFC resulting in reduced selective attention to threat during experimentally induced anxiety. This is especially interesting in conjunction with the results of the latency data, which revealed that the active group was slower to attend to the threat valenced stimuli on the pro-saccade trials.

There is evidence of similar findings of reduced selective attention to threat in studies using other brain stimulation modalities and other emotional attentional tasks. For example De Raedt et al (2010) stimulated the left DLPFC using high frequency rTMS and examined changes in attentional processing of emotional information using an emotional modification of an exogenous cueing task²⁴ during event-related fMRI: and found that the stimulation of the left DLPFC resulted in diminished attentional engagement by angry faces, which was contrasted by stimulation of the right DLPFC which resulted in impaired disengagement from angry faces. Collectively these results

²⁴ Exogenous cueing paradigm of Posner (1980) requires participants to detect a visual target presented at the left or right side of a fixation cross. On most of the trials a cue precedes the target at the same spatial location (valid trials) and on the other trials the target is presented at the opposite spatial location to the cue (invalid trials). In the modified version the emotional value of the cue is varied (threat/neutral) and both engagement to threat (RTs on valid trials, threat vs neutral) and disengagement from threat (RTs on invalid trials threat vs neutral) can be studied (Koster et al, 2005).

suggest that stimulation of left DLPFC by rTMS or modulation by anodal tDCS can result in more accurate disengagement from threatening stimuli using a variety of emotional attentional tasks.

No published studies using tDCS and an Antisaccade Task investigating hypervigilance to threat were identified for a direct comparison of current findings.

Latency

The results from the latency analysis indicate that both groups performed faster on pro- as compared to anti-saccade trials and tDCS did not modify the RTs. However there was a strong trend for the active tDCS group to be slower to attend to the threat stimuli in the pro-saccade trials relative to the sham group (slower engagement to threat). This is an interesting result, as one of this study predictions was that active anodal tDCS would result in faster (correct) latencies on antisaccade trials (faster disengagement from threat). Even though this was not observed, this study provides some support to the notion that active anodal tDCS can change patterns of attention to threat (latency results) as well as away from threat (accuracy results).

Another study has examined the effects of tDCS on the antisaccade task - Kanai et al (2012) described in section 3.1, which although interesting, a) stimulated an area not typically implicated in anxiety and b) primarily investigated the effects of tDCS on the saccade tasks, whereas in the context of anxiety, the antisaccade task is used as a tool to measure attentional deficits present in anxiety, which need to be targeted. Due to the lack of directly comparable literature, evidence was gathered looking at the effects of another stimulation technique – TMS – on task performance. A study by Nagel et al (2008) used single pulse TMS applied to the DLPFC or FEF in a sample of 10 healthy participants. The authors found that TMS to DLPFC or FEF increased the latencies of both pro- and antisaccades. Moreover TMS-induced increase in saccade latency was stronger with pro-saccades than with antisaccades. This finding accords with the current latency finding, suggesting that active tDCS of DLPFC can have a slowing effect on the initiation of pro-saccades in the saccade task.

3.4.4 The Sample

The current study sample comprised healthy volunteers who did not have clinical levels of anxiety which may have had an effect regarding the tDCS effects. A body of evidence suggests that tDCS, which is a relatively ‘weak’ form of brain modulation, works only on a brain that does not ‘perform’ optimally - such as in patients suffering from various conditions (e.g. Batsikadze et al, 2013). Batsikadze et al (2013) proposed that healthy subjects may have a ‘ceiling effect’ in a single administration protocol and that repeated administration protocols might be more effective to

enhance the efficacy of stimulation (Monte-Silva et al. 2010). This idea of multiple sessions is especially cogent taking into account other studies which suggest that tDCS impacts cognition via repeated exposure and, possibly, overnight consolidation (e.g. Marshall et al, 2011). In the current study the participants were a) healthy, b) underwent a single tDCS administration protocol and c) underwent tDCS prior to undergoing 7.5% CO₂ challenge meaning that perhaps the effects were not as robust as they could have been if tDCS was administered either during the inhalation or repeatedly over a period of time; or in a sample of clinically anxious individuals.

A recent study by Clarke et al (2014) (see Chapter 1, section 1.6.2.5) which aimed to ascertain experimentally the role of DLPFC in modification of attentional bias by delivering tDCS during attention bias modification training provides a good example to show that tDCS should be administered during the experimental task and not prior to it. Clarke et al (2014) found that participants receiving active tDCS showed greater evidence of attentional bias acquisition in the targeted direction (toward or away from threat) compared with participants in the sham condition and the participants began tDCS from the time they initiated the attentional probe practice trials until the break at the midpoint of the task.

The after effects of tDCS depend on the length of the administration, with a 5-7 minute single administration tDCS having after-effects reported at no longer than 5 minutes, and administration durations of 9-13 minutes resulting in after-effects of 30 to 90 minutes respectively (Nitsche & Paulus, 2001) thus there is a reasonable assumption that in the current study the participants experienced after effects of tDCS whilst undergoing 7.5% CO₂ challenge and the subsequent antisaccade task, even though they did not experience the immediate effects.

3.4.5 Future Directions

Should this study be replicated there are a few methodological changes that could be considered. Even though there is a reasonable assumption in the current protocol that the participants in the active tDCS group are experiencing positive after effects of tDCS as the administration duration was 20 minutes, the participants were however not experiencing the immediate effects of the tDCS when performing the experimental task measuring the attentional bias. Beginning the experimental task half-way through the tDCS administration duration could be considered to address this issue.

Moreover, as the tDCS administration occurred at the beginning of the experimental session, prior to the 7.5% CO₂ challenge, tDCS is applied to a brain in a healthy, non-anxious state (rather than in a high state-anxiety state), and thus may not be as effective as if the brain was stimulated at the same time as anxiety was induced. If the 7.5% CO₂ model of anxiety is a reliable model for

generalized anxiety, it is reasonable to predict that it would alter the pattern of brain activation from pre-inhalation (at rest, healthy brain) to during the inhalation when the healthy brain may display a pattern of activation typically observed in patients with GAD and in that case it would be more beneficial perhaps to stimulate the brain when in the anxious state.

In the current study the participants were healthy volunteers who underwent a single tDCS administration protocol prior to undergoing the 7.5% CO₂ challenge and in future studies could try to manipulate these variables when searching for the optimal application of tDCS in the treatment of anxiety.

iv. Chapter 4

Effects of Attention Training (ABMT) on the Subjective, Autonomic and Neuropsychological Response to the 7.5% CO₂ Challenge.

4.1 Introduction

Remission rates in first line treatments for anxiety disorders (CBT and pharmacotherapy) are around 50% (Ballenger, 2004; Barlow, Gorman, Shear, & Woods, 2000; Cartwright-Hatton, et al, 2004), thus more efficacious treatments are required. Attentional Bias Modification Training (AMBT) (see Chapter 1, section 1.6.1) is a novel treatment option that stems from established experimental studies of threat-related attentional biases in anxiety (for a review see Bar-Haim, et al, 2007). These information-processing biases are suggested to play a central role in the aetiology and maintenance of anxiety disorders (Beck & Clark, 1997; Eysenck, 1992, 1997; Rapee & Heimberg, 1997; Williams, Watts, MacLeod, & Mathews, 1997). Thus the premise of ABMT is to use computer-based attention training to modify biased attentional patterns in anxious patients and subsequently reduce anxiety (Bar-Haim, 2010).

CBT – a first line treatment (see section 1.5.1) – focuses primarily on “training in relaxation techniques, systematic and graded exposure to threat as a vehicle promoting fear extinction, and direct, explicit, and conscious challenging of negative interpretive and memory biases” (Bar-Haim, 2010, p.859). ABMT extends CBT through directly and implicitly targeting early, automatic and sometimes unconscious attentional biases. ABMT does not explicitly teach anxious individuals top-down effortful attention control like CBT, and instead addresses biases in attention allocation that are mediated by functional interactions between subcortical and cortical neural circuits that are not necessarily available to conscious regulation (e.g. Delgado, Nearing, LeDoux, & Phelps, 2008; LeDoux, 2000; Pine, 2007).

In standard protocols attention is modified using a standard dot probe test (MacLeod, Mathews, & Tata, 1986) (see Chapter 1, section 1.4.1). In brief, in each trial of a standard dot probe task two stimuli (one threat-related and one neutral) are briefly shown, and their removal is followed by a small target probe in the location just vacated by one of the stimuli. Participants are required to discriminate as quickly and accurately as possible between two variants of a probe. In the classic version of this task targets appear with equal probability at the location of threat and neutral stimuli, measuring the distribution of participants’ attention - attentional bias towards threat is

revealed when participants are faster to respond to probes that replace threat-related stimuli rather than neutral stimuli. ABMT is a modified version of the classic dot probe task, where the probability of the probe appearing at the location previously occupied by neutral stimuli is much higher than it appearing at the location previously occupied by the threatening stimuli (e.g. 90% or 100%). Over time this encourages participants to selectively prioritise attention to neutral over threat stimuli, as evidenced by faster reaction times to neutral probes after compared to before training.

4.1.1 Evidence of effectiveness

Recent evidence suggests that ABMT is effective in reducing selective attention for threat, and in subsequently reducing levels of anxiety vulnerability (Macleod & Clarke, 2015).

In 2002 MacLeod et al demonstrated that it is possible to modify patterns of attention to threat in non-anxious participants and that such changes alter affective responses to subsequent stress. MacLeod et al (2002) used a variant of the dot-probe task to train non-anxious participants either to adopt a biased attentional response toward threat words (i.e. probes always appeared in the loci of threat) or to adopt an attentional bias away from threat (i.e. probes always appeared in the loci of neutral words). They found that these two groups of participants developed differentially biased attention responses that were in accord with the assigned threat-target contingency. Despite the fact that this differential attention bias did not result in an immediate change in anxiety following training (participants all had low levels of trait anxiety), attention modulation did lead to a group difference in anxiety vulnerability to a subsequently induced stress using a standardized stress task. Both groups responded to the stress manipulation (stressful anagram task) with elevation in state anxiety; however the magnitude of this elevation was significantly greater in participants who had been trained to attend to threat as compared to neutral stimuli. This study provides evidence that training selective attention to negative information increases anxiety reactivity to an experimental stressor.

Similarly, Amir, Weber, Beard, Bomyea, and Taylor (2008) exposed socially anxious undergraduate students to a single dot-probe session (128 critical trials) of either attention training away from facial expressions of disgust or a control condition. Relative to participants who received control placebo training, participants who were trained to attend away from disgust faces showed significantly less attention bias after training as well as lower levels of anxiety in response to a public-speaking challenge.

Recently, Heeren, Reese, McNally, and Philippot (2012) have replicated these observations and extended these findings to examine the effect of training on sympathetic activation to stressors

occurring after the training. They reported that attention training reduced skin conductance reactivity to an impromptu speech stressor. These results support the effectiveness of ABMT in both reducing the attentional threat bias and in reducing the autonomic and subjective characteristics of anxiety.

The use of ABMT has extended from sub-clinical, non-anxious samples to use within clinical samples (see Clarke et al, 2014 for a review). Three early studies provided compelling evidence that ABMT can attenuate the symptoms of patients suffering from anxiety disorders. In a randomized controlled trial, Schmidt Richey, Buckner, and Timpano (2009) assigned patients with generalized social phobia to either a training condition designed to reduce vigilance for threat (using disgusted facial expressions as stimuli) or to a control condition. In this study patients received eight sessions of ABMT across four weeks. After the intervention, 72% of the patients in the attentional training condition no longer met diagnostic criteria for social phobia, compared to 11% of the patients in the control condition. At follow-up assessment four months later, the therapeutic gains were maintained, with 64% of the participants in the attentional training condition classified as remitted compared to only 25% of those in the control condition.

Amir and colleagues (2009a) using the same procedure as Schmidt et al (2009) found that in a group of patients diagnosed with generalized social phobia, the percentage of participants no longer meeting DSM-IV criteria for their condition post treatment was 50% in the active ABMT training group, compared to only 14% in the placebo control group.

The third early clinical study was conducted by Amir, Beard, Burns, and Bomyea (2009b) and examined the effects of ABMT in patients with generalised anxiety disorder (GAD). The researchers randomly allocated patients with GAD to either an attentional training condition designed to induce avoidance of threat words or to an attentional control condition. This dot-probe training entailed 160 trials of threat-neutral word pairs per session, administered in eight sessions across a four-week period. Following training 58% of the patients in the attentional training condition no longer met DSM-IV diagnostic criteria for GAD compared to only 17% of the patients in the control condition. The clinical efficacy of the attentional training procedure was further supported by improvements on a range of clinician-delivered and self-report measures of anxiety.

More recently Heeren et al (2011) demonstrated in a sample of participants diagnosed with social phobia that learning to disengage attention from threat is crucial in successful attentional training. In this experiment, patients with social phobia were randomly assigned to one of four training conditions: (1) disengagement from threat, (2) engagement towards non-threat, (3) disengagement from threat and re-engagement towards non-threat, and (4) a control condition.

Effects were examined on subjective and behavioural responses (e.g, VAS; modified Posner spatial cueing task) to a subsequent stressor (speech task). Data revealed that training to disengage from threat reduces behavioural indices of anxiety. Engagement towards non-threat faces did not have effects in itself. These results support that the difficulty in disengaging attention from threat is a critical process in successful reduction of an attentional bias to threat.

Subsequent studies have also shown promising results in paediatric anxiety disorders using home-based delivery of ABMT. Waters et al (2013) found that two weeks (12 sessions) of ABMT completed by children with clinical anxiety at home resulted in 50% of those in active training no longer meeting diagnostic criteria compared to 8% in the control condition.

Despite these positive results, some recent studies have not found evidence of clinical benefits of ABMT under specific conditions. Carlbring and colleagues (Carlbring et al, 2012) did not modify biased attention and anxiety symptoms among a group of socially anxious individuals. At the time of its publication, the study by Carlbring et al. (2012) represented the first study to have implemented an ABMT task without therapeutic success within a controlled trial. It is important to note that the ABMT training and the control condition were both administered over the Internet rather than in a lab setting, which may pose difficulties with adherence to therapy. There have since been a number of other studies that have delivered attentional bias modification tasks that have neither successfully modified biased attention nor impacted clinical symptomology. Several of these have targeted social anxiety disorder (Boettcher et al, 2012; Boettcher et al, 2013; Neubauer et al, 2013) - interestingly all also delivered over the Internet - with another unsuccessfully attempting to modify biased attention in sufferers of posttraumatic stress disorder (Schoorl et al, 2013) with treatment sessions also taking place over the Internet. The emerging picture from these unsuccessful trials indicate that ABMT potentially may not be as effective when delivered over the Internet rather than in person, and moreover critically, none of these studies succeeded in modifying attentional bias (i.e. it is not the case that biased attention was successfully altered but this failed to influence measures of emotional vulnerability, but instead these studies failed to alter patterns of selective attention in the first place).

Indeed, “the very fact that the studies which have failed to modify selective attention have also failed to modify emotional vulnerability provides reassurance that the theoretical basis for ABM is sound” (Clarke et al, 2014, p.3). Clarke et al (2014) reviewed 42 ABM studies (39 papers) and found that 16 identified both an attentional bias modification and a subsequent change in emotional vulnerability, and 10 observed neither a bias modification nor an emotional change. Taken together these results were consistent with the theory that when attentional bias is achieved so emotional vulnerability changes.

Two meta-analyses have been conducted providing some general insight into the therapeutic potential of ABM (Hakamata et al, 2010; Hallion & Ruscio, 2011) (see chapter 1, section 1.6.1). Hallion and Ruscio (2011) pooled the results of both attentional and interpretive bias modification studies and reported small estimates of effect sizes ($g = 0.13 - 0.29$), however the meta-analysis and its methods have been criticised (e.g. Clarke et al, 2014). In contrast, Hakamata, et al. (2010) included attentional bias modification studies exclusively, and reported estimated effect sizes in the medium range ($d = 0.61$). Largest effect sizes were reported amongst patient populations ($d = 0.78$) (non-clinical $d = 0.48$), comparing favourably with traditional psychological interventions ($d = 0.86$) (Powers et al, 2008). In sum, meta-analyses suggest that ABMT has promise as a therapeutic intervention for clinical and subclinical anxiety (Hakamata et al, 2010).

4.1.2 Extending to different tasks/generalizability

This study aimed to test the effectiveness of a single session of ABMT on resiliency to a subsequent stressor – using the 7.5% CO₂ model of anxiety (see Chapter 1, section 1.7.1). Evidence suggests that the 7.5% CO₂ challenge is a useful translational model, which can temporarily induce symptoms typically displayed by clinically anxious populations (subjective self-report anxiety, elevated autonomic arousal and attentional disturbances) (e.g. Bailey et al. 2005; Bailey and Nutt 2008; Bailey et al. 2011a; Poma et al. 2005; Garner et al, 2011).

Direct measurement of a bias is needed in order to confirm that ABMT modifies it as intended (if a therapeutic impact is achieved without a significant modification of the bias, then attributing the therapeutic effect to the alteration of selective processing via ABMT cannot be stated). Thus MacLeod, Koster and Fox (2009) recommend a routine inclusion of a performance-based measure of the bias in ABMT studies. Published studies vary with respect to this, with some reporting it (e.g. Amir et al, 2009; Holmes et al, 2009; Joormann et al, 2009), some relying on self-report measures to infer such change (e.g. Schartau et al, 2009; Watkins et al, 2009) and some report no measures of the cognitive bias that the ABMT was designed to modify (Hirsch et al, 2009; Schmidt et al, 2009).

In order for an ABMT manipulation to affect real-life experience (by influencing the processing of naturalistic situations), the induced change in attentional bias must be generalizable across different circumstances and not be shown only within the ABMT task itself (MacLeod, Koster & Fox, 2009). The majority of previous studies report assessing the ‘transfer’ of attention bias modification to other aspects of cognition and emotion by using stimulus materials not previously employed in the training trials (e.g. Amir et al, 2009; See et al, 2009). However, rarely investigators appraise the impact of training using a bias assessment task markedly different from

the training task (i.e. not just new stimuli such as different words or pictures but different tasks altogether). The routine use of measures of cross-task transfer of training effects is highly recommended in order to ensure generality of induced cognitive change (MacLeod, Koster & Fox, 2009).

The aim of this current study was to investigate whether the attentional training using ABMT can extend from a classical dot probe task to other behavioural tasks designed to measure attentional bias. Healthy volunteers were randomised to either receive 20 minutes of ABMT or control condition (standard dot probe task) prior to undergoing a 20 minute CO₂ challenge, designed to elevate their anxiety levels. During the challenge all participants were asked to complete an antisaccade task (Hallet, 1978) (see Chapter 1 section 1.4.2). This task was chosen in order to examine transfer effects from the ABMT training (modified dot probe task, word stimuli) to a different experimental task measuring attentional bias (using picture stimuli). The Antisaccade Task extends visual probe measures of attentional bias by a) providing a control task (the pro-saccade task), and b) by providing two distinct performance measures: effectiveness (i.e. error rate), and processing efficiency (i.e. latencies) (Kristjansson et al., 2001; Eysenck et al., 2007). The use of the antisaccade task to investigate the effects of anxiety on attentional control has been suggested by Miyake et al (2000).

The study hypotheses examined in this chapter are that:

HA1: a single session of 20 minutes of ABMT in healthy individuals will improve the performance (accuracy and latency) on the Antisaccade Task during CO₂ challenge (compared to standard dot probe task).

HA2: a single session of 20 minutes of ABMT in healthy individuals will improve self-reported mood and anxiety levels post anxiety-induction.

4.2 Method

4.2.1 Design

A between-subjects design compared two groups (active ABMT group versus Control) on post-training measures of subjective anxiety, autonomic arousal and antisaccade performance during 7.5% CO₂-challenge.

4.2.2 Participants

Thirty eligible participants were screened for physical and mental wellbeing and randomized to receive either 20 minute of active ABMT; $n = 15$; 8 females; mean age = 20.47 years ($SD=3.00$) or control condition (standard dot probe task); $n = 15$; 7 females; mean age = 20.87 years ($SD=2.89$). The study randomisation was double-blind. The two versions of the dot probe task (standard = control; modified = training) were labelled as “condition A” and “condition B” by an independent researcher not involved in data collection. The status of the conditions (active vs inactive) was revealed to the experimenter only upon completion of the data collection phase of the study. The screening procedure and exclusion criteria were identical to those described in Chapter 2 (section 2.2). In brief, all participants were healthy volunteers who were required to be aged 18-55 years. Eligibility was assessed using a phone health interview and again as part of the pre-test diagnostic interview (Mini International Neuropsychiatric Interview MINI) (Sheehan et al., 1998).

Exclusion criteria for this study were: metal or electronic implants, epilepsy, currently pregnant or breastfeeding (self-report), history of mental health illness including anxiety and depression, high blood pressure ($>140/90$), use of medication in the past 8 weeks other than local treatments, paracetamol/aspirin or contraceptives, and a Body Mass Index (BMI) of < 18 , or >28 . Participants' alcohol intake was required to not exceed 50 units per week (for males) and 35 units per week (for females).

Recruitment and screening procedures and test protocols were approved by the Research Ethics and Governance Committee at the University of Southampton. Study Information, Consent and Debrief forms are presented in Appendices (A5; A11-A12).

A power calculation was performed a priori for a repeated measures ANOVA with between-within interactions with 3 factors (group x trial type x valence) which revealed that the sample size needed to detect a modest effect size ($\eta^2P = 0.15$) with the alpha level of .05 and high power of 0.8 was $n=30$ (15 v 15). An effect size of at least this magnitude was expected, informed by the results of previous 7.5% CO_2 studies (e.g. Ainsworth et al, 2015 reports $\eta^2P = .27$ for saccade accuracy). In terms of effect sizes in other ABMT studies, a review by Hakamata et al (2011) generated an averaged effect size across 12 datasets and found a medium effect size of ABMT on anxiety measures ($d=0.61$).

4.2.3 Materials

This study used all measures reported in Chapter 2 except the GAD7 (section 2.2). In brief, these included measures of trait anxiety (STAI, PSWQ, ASI), attention control (ACS), state mood (PANAS, SSAI, VAS) and heart rate and blood pressure. Please see Appendix A6 for scales.

Tasks

ABMT

The ABMT task used in this study was identical to that used by Hayes et al (2010) and used a set of 96 threat–nonthreat word pairs (e.g. afraid – detail; MacLeod et al, 2002). Two sets were created, and matched in length and frequency of usage: one for training (with the target consistently in the location of the nonthreat word) and one for test (standard dot probe, target in the location of each word type with a 50% frequency). All training trials began with a fixation cross followed by a threat–nonthreat word pair (one above and one below fixation) and after 750 ms, one word was replaced by a single or double dot target. Participants were required to respond as quickly as possible by pressing an appropriate key, and given feedback (“correct response” or a tone signalling error). 480 training trials were divided into 10 blocks of 48 with eight test trials per block, preceded by two training trials.

Antisaccade Task

This task was identical to that described in detail in Chapter 3 (section 3.2). In brief on each trial, a word (either ‘TOWARDS’ or ‘AWAY’) was presented at central fixation for 2000 ms and instructed participants to look towards (prosaccade) or away from (antisaccade) the picture stimulus. At 200 ms following word offset, the picture stimulus was presented for 600 ms (6° to the left or right of central fixation). On pro-saccade trials, participants were required to look toward the picture and on antisaccade trials to look away from it. There were 96 fully randomized experimental trials.

Stimuli were presented using Inquisit 2 Computer software, Millisecond Software, Seattle, WA. Horizontal eye movements were measured by electrooculography and sampled at 1000 Hz (MP150-amplifier and AcqKnowledge-3.8.1 software, Biopac-Systems, Goleta, CA).

4.2.4 Procedure

Consistent with the method employed in Chapters 2 and 3, participants responded to a study advert and were telephone screened. Eligible participants were instructed to refrain from alcohol and caffeine consumption for 24 hours prior to the test session. On the study day participants

provided informed consent and underwent further screening with the MINI and blood pressure/heart rate. Testing was completed in testing lab in the Academic Unit of Psychology with controlled lighting and temperature. Eligible participants completed trait measures (STAI, ASI, ACS, and PSWQ) and baseline state measures of anxiety and mood (SSAI, PANAS and VAS).

Baseline measures of blood pressure and heart rate were taken.

CO₂ challenge

The procedure for the preparation and administration of the 7.5% CO₂ challenge was identical to that described in Chapter 3 (section 3.2). An oral-nasal face mask (see Appendix 3G) was fitted onto a participant's face, and connected via a plastic tube to an inflatable bag attached to the canister containing the premixed gas mixture. Participants were asked to breathe through the mask as normal. Midway through the inhalation participants began the Antisaccade Task. Immediately following the 20 minute inhalation participants again completed state measures: VAS, PANAS and SSAI as well as blood pressure and heart rate measures. Finally, participants were asked to complete a final set of supplementary questionnaires, including the Attention Control Scale (ACS) (Derryberry & Reed, 2002) and Penn State Worry Questionnaire (PSWQ) (Meyer et al, 1990). The test session lasted approximately 3 hours. Participants were debriefed and contacted 24 hours later to discuss any further queries and register any side effects; none were reported.

4.3 Results

4.3.1 Group Characteristics

Data from 30 participants were collected and included in the analysis. Independent samples t-tests indicate that active ABMT and control groups did not significantly differ on measures of pre-existing trait anxiety or demographic characteristics, however they did significantly differ on their trait attention control (ACS) ($t(1,28)=2.07, p=.05$)²⁵. For group characteristics see Table 14.

²⁵ The main analyses (Antisaccade Task accuracy and latency) were repeated including ACS as a covariate, with no impact on any of the group effects nor revealing any significant ACS interactions ($F's(1,26)<2.75, p's>.12$).

Table 14

Group Characteristics for Active and Control ABM Training.

	Active ABMT (n=15)		Control (n=15)		t(28) =
	M	SD	M	SD	
STAI-trait ²⁶	34.07	6.94	31.73	4.30	1.11, p=.28
ACS	48.31	7.87	54.57	7.83	-2.07, p=.05*
ASI	33.60	8.86	32.13	7.40	0.49, p=.63
PSWQ	44.57	14.47	38.07	8.50	1.45, p=.16
Age	20.47	3.0	20.87	2.72	-0.38, p=.71
BMI	22.97	2.29	21.95	2.89	1.07, p=.29
Gender (m:f)	7:8		8:7		

ACS = Attention Control Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire

²⁶ Please note that despite this study being presented as third out of four conducted, chronologically it had been started as first, and the lack of use of GAD 7 here is not a discontinuation but a lack of consideration to use it as a continuous measure at that early stage of the thesis.

4.3.2 Threat Bias

An attentional bias score reflects the extent to which attention is preferentially allocated to threat relative to neutral stimuli. In the visual probe task this is calculated as (RT_threat_incong minus RT_threat_cong), whereby a large positive score reflects faster RTs to probes that appear behind threat relative to neutral stimuli (i.e. vigilance for threat), a negative score reflects avoidance of threat, and zero reflects no bias.

A mixed design analysis of variance (ANOVA) (2 x 2) with a between-subjects factor of group (active ABMT versus control) and a within-subjects factor of time (bias score in first vs. final block) revealed no main effect of time, no main effect of group and no time x group interaction ($F's(1,26) < 1.09, p's > .31$). Consequently there was no evidence that this ABMT successfully trained attention away from threat. Potential reasons thereof will be considered in the Discussion section.

Four one-sample t-tests compared the four bias scores (Table 15) against zero (no bias) revealing that none differed significantly from a no bias score ($t's(14) < 1.31, p's > .21$).

Table 15

Threat Bias Change over Time

		Active ABMT		Sham ABMT		
		n=15		n=15		
		M	SD	M	SD	t(28) =
Threat bias	Start	1.86	31.09	-1.91	20.02	0.40, p=.70
Threat bias	End	1.99	34.02	-10.31	33.66	1.01, p=.32

4.3.3 Anxiety and Mood

A mixed design analysis of variance (ANOVA) (2 x 3) with a between-subjects factor of group (active versus sham tDCS) and a within-subjects factor of time (baseline, post-training, post- CO₂) revealed no main effects of group in all of the repeated self-report mood and anxiety measures ($F's (1,28) < 1.42, p's > .24$) and no significant group x time interactions ($F's (1,28) < 0.58, p's > .57$).

Results revealed a significant main effect of time for all bar one of the repeated self-report anxiety and mood measures: SSAI ($F(1,28)=40.60, p < .001, \eta^2P=0.60$), PANAS negative affect

($F(1,28)=5.16, p=.03, \eta^2P=0.16$), VAS negative affect ($F(1,28)=5.42, p<.01, \eta^2P=0.16$), VAS positive affect ($F(1,28)=12.86, p<.001, \eta^2P=0.32$) and VAS cognition ($F(1,28)=7.38, p<.01, \eta^2P=0.21$). For PANAS positive affect there was a trend in the same direction as other measures ($F(1,28)=2.95, p=0.10, \eta^2P=0.10$).

Paired comparisons between each time point (collapsed across group) revealed significant increases in anxiety and negative affect, and decreases in positive affect, following CO₂-challenge (relative to baseline and post-training), but no differences between baseline and post-task for SSAI, PANAS negative affect, and VAS positive affect (p 's < .05, see superscripts in Table 16). For PANAS positive affect there were no significant differences between the time points across groups, however significant decreases in positive affect were observed post-CO₂ in the control group only. Similarly for VAS negative affect no significant changes were observed between the time points in the active group, but in the control group negative affect increased from baseline to post-training, and increased further post- CO₂ with a significant difference between baseline and post-CO₂ only. For VAS cognition across groups alertness, attentiveness and mindfulness dropped significantly post-training but did not decrease further post- CO₂. This suggests that a single session of active ABM training does not modify mood or subjective anxiety levels, or the anxiogenic response to CO₂-challenge, however both training conditions resulted in dampened cognition.

Table 16

Mean Measures of Self-Report Anxiety and Mood at Different Time Points.

		Active ABMT n=15		Sham ABMT n=15		t(28) =
		M	SD	M	SD	
SSAI	Baseline	30.07 ^a	6.94	30.40 ^a	4.26	0.06, p= .95
	Post-Inhalation	40.79 ^b	11.64	42.73 ^b	11.02	-0.46, p=.65
PANAS positive	Baseline	31.43 ^a	7.77	30.80 ^a	8.39	0.14, p=.89
	Post-Inhalation	30.07 ^a	16.28	25.53 ^b	9.37	0.93, p=.36
PANAS negative	Baseline	13.47 ^a	4.22	12.20 ^a	2.91	0.96, p=.35
	Post-Inhalation	14.80 ^a	5.19	15.40 ^a	6.56	-0.28, p=.78
VAS negative	Baseline	36.98 ^a	31.50	27.18 ^a	20.26	1.01, p=.32
	Post-Training	41.02 ^a	29.60	39.04 ^{ab}	24.62	1.21, p=.24
	Post-Inhalation	50.07 ^a	37.50	45.38 ^b	36.77	0.35, p=.73
VAS positive affect	Baseline	97.77 ^a	20.44	91.33 ^a	21.76	0.84, p=.41
	Post-Training	91.37 ^a	27.42	80.83 ^a	31.97	0.97, p=.34

	Post-Inhalation	77.63 ^b	32.19	63.53 ^b	32.88	1.19, p=.25
VAS cognition	Baseline	97.24 ^a	22.97	94.22 ^a	23.77	0.35, p= .73
	Post-Training	86.84 ^b	21.38	77.73 ^b	31.80	0.92, p=.37
	Post-Inhalation	88.62 ^b	17.67	77.51 ^b	33.08	1.15, p=.26

Spielberger Trait – State Anxiety Inventory; Positive and Negative Affect Scale; Visual Analogue Scale

Within each measure and group values with different superscripts significantly differ, $p < .05$

4.3.4 Autonomic Arousal Changes

Active ABM training did not modify autonomic measures (heart rate, systolic and diastolic blood pressure) as compared to control training (see Table 17). ANOVA (2x3) of heart rate revealed a significant main effect of time ($F(1,28)=10.43, p<.001, \eta^2P=0.21$). There were no significant effects of group or time x group interaction ($F's(1,28)<0.60, p's>.55$). Paired comparisons between each time point (collapsed across group) revealed significant increases in HR following CO₂ -challenge (relative to post-training), but no differences between baseline and post-training ($p's < .05$, see superscripts in Table 17). No significant main effect of group nor a time group x interaction were observed ($F's(1,28)<0.60, p>.55$).

A corresponding ANOVA of systolic blood pressure (SBP) revealed similar results, with a significant main effect of time ($F(1,28)=10.92, p<.001, \eta^2P=0.29$). Paired comparisons between each time point (collapsed across group) revealed significant increases in SBP following CO₂ -challenge (relative to post-training), and also significant decreases in SBP between baseline and post-training ($p's < .05$, see superscripts in Table 17). No significant main effect of group nor a time group x interaction were observed ($F's(1,28)<0.10, p's>.88$).

The ANOVA of diastolic blood pressure (DBP) also revealed a significant main effect of time ($F(1,28)=7.40, p<.01, \eta^2P=0.16$). No main effect of group and no time x group interaction were observed ($F's(1,28)<0.20, p's>.82$). Paired comparisons between each time point (collapsed across group) revealed significant increases in DBP following CO₂ -challenge (relative to post-training). Upon further investigation this increase was only significant in the control group.

Together these results suggest that that a single session of attentional bias modification training does not alter blood pressure and heart rate measures of autonomic arousal (neither immediately after training nor in response to CO₂ challenge). However a single 20 minutes inhalation of the 7.5% CO₂ mixture does induce anxiety as measured by autonomic arousal, which validates the experimental model.

Table 17

Heart Rate, Systolic Blood Pressure, and Diastolic Blood Pressure Mean Measures at Different Time Points.

		Active ABMT		Control		
		n=15		n=15		
		M	SD	M	SD	t (28) =
HR	Baseline	70.20 ^a	8.77	70.33 ^{ab}	12.10	-0.04, p=.97
	Post-Training	71.60 ^a	9.54	67.80 ^b	12.47	0.94, p=.36
	Post-Inhalation	78.67 ^b	7.93	75.93 ^a	17.01	0.56, p=.58
Systolic BP	Baseline	122.27 ^a	17.25	121.47 ^a	12.03	0.16, p=.87
	Post-Training	116.27 ^b	17.02	116.40 ^b	16.26	-0.03, p=.98
	Post-Inhalation	126.13 ^a	17.81	124.53 ^a	15.28	0.26, p=.79
Diastolic BP	Baseline	69.93 ^a	9.41	69.33 ^{ab}	6.60	0.14, p=.89
	Post-Training	69.00 ^a	7.22	67.93 ^a	6.28	0.43, p=.67
	Post-Inhalation	72.20 ^a	8.63	72.13 ^b	5.91	0.03, p=.98

Within each measure and group values with different superscripts significantly differ, $p < .05$

4.3.5 Antisaccade Results

Antisaccade Task

The task was identical to that described in detail in Chapter 3 (section 3.3.4). In brief, saccade direction and latency were scored manually and blind to trial type and inhalation condition using AcqKnowledge software and saccade accuracy and latencies for correct saccades were entered into separate repeated-measures analysis of variance (ANOVA) with group (Active ABMT vs Control), trial type (pro vs antisaccade), and image valence (negative vs neutral) as independent variables.

Prior to conducting the main analyses, data from the 30 participants was screened for presence of outliers. Two cases were excluded from any further analyses following this procedure. One subject (active group) was considered an outlier on accuracy and latency measures for both negatively and neutrally valenced pro-saccades (pro-negative latency = 230.60, pro-neutral accuracy = 305.52, pro-negative accuracy = .46 and pro-neutral accuracy = .33). This subject displayed both the longest latencies as well as the highest error rates on those trials. The second excluded subject (control group) and was an outlier on latency measures for antisaccade trials (anti-negative = 453.00; anti-neutral = 513.00). The following analyses included data from the 28 remaining subjects.

Accuracy

Table 18 below shows error rates (ER) for all the 4 trial type - valence combinations split by group (ABMT vs Control). A group (2) by cue (pro- versus anti-saccade) (2) by valence (threat versus neutral) (2) ANOVA revealed a significant main effect of cue type characterised by overall lower ERs for pro ($m = 0.05$) as compared to antisaccade trials ($m = 0.53$) ($F(1,26)=84.83$, $p<.001$, $\eta^2P=0.76$) No other significant main effects or interactions emerged (F 's (1,26) <1.78 , p 's $>.19$).

The results from the accuracy analysis indicate that both groups were significantly more accurate on pro- as compared to anti-saccade trials and ABMT did not result in reduced selective attention to threat during experimentally induced anxiety.

Table 18

Antisaccade Accuracy Data

		Active ABMT		Control		t(26) =
		n=14		n=14		
		M	SD	M	SD	
Antisaccade	Neutral	0.56	0.25	0.50	0.29	0.59, p=.59
	Negative	0.50	0.24	0.50	0.28	-0.03, p=.98
Prosaccade	Neutral	0.05	0.08	0.04	0.05	0.41, p=.69
	Negative	0.04	0.04	0.03	0.08	0.29, p=.78

Latency

Table 19 below shows mean reaction times for all the 4 trial type - valence combinations split by group (ABMT v Control). A group (2) by cue (pro- versus anti-saccade) (2) by valence (threat versus neutral) (2) ANOVA revealed a significant main effect of cue type characterised by overall faster reaction times (RTs) for pro ($m = 179.54\text{ms}$) as compared to antisaccade trials ($m = 277.00\text{ms}$) ($F(1,26)=112.31, p<.001, \eta^2P=0.74$) but no other significant main effects or interactions (F 's (1,26) < 0.96, p 's > .34).

The results from the latency analysis indicate that both groups performed faster on pro- as compared to anti-saccade trials and ABMT did not modify the latencies of pro nor anti-saccades to negative or neutral stimuli.

Table 19

Mean Reaction Times for the Active and Control ABMT Conditions by Trial Type and Valence (ms)

		Active ABMT		Control		t(26) =
		n=14		n=14		
		M	SD	M	SD	
Antisaccade	Neutral	294.37	80.96	282.93	85.99	0.33, p=.75
	Negative	271.90	75.01	279.31	74.84	-0.25, p=.81
Prosaccade	Neutral	173.33	23.60	181.44	13.09	-0.87, p=.39
	Negative	174.45	21.95	182.17	28.64	-0.78 p=.44

4.3.6 Blinding / Expectancy

Data on retro-expectancy was not collected for this study. Literature suggests that majority of participants in both active and control conditions in ABM protocols believe that they did not receive an active treatment (79% in the control condition vs 72% in the ABM condition) with no significant differences in this awareness between conditions (e.g. Amir et al, 2009). A retro-expectancy of similar direction was expected to be true for both groups in Study Three.

4.4 Discussion

4.4.1 Summary of results

Consistent with previous studies, inhalation of the 7.5% CO₂ mixture produced robust effects on subjective anxiety levels and mood characterised by significant increases in anxiety and negative affect, and decreases in positive affect, following the CO₂ challenge (relative to baseline and post-training). The inhalation also led to significant increases in autonomic arousal characterised by significant increases in heart rate and blood pressure post inhalation thus suggesting that the challenge successfully induced subjective and autonomic anxiety (similarly to findings of Study Two, Chapter 3).

Unlike a number of other studies (e.g. Hayes et al, 2010; Amir et al, 2009a, 2009b) the results of Study Three indicated that ABMT did not train attention (i.e. it had no effect on the threat bias). Consequently it is not surprising that ABMT did not modify the response to the CO₂ model of anxiety (i.e. there was a failure to train bias). The task used in Study Three was a replication of that used by Hayes et al (2010), which did successfully train the attentional bias. Possible reasons for the disparity in findings will be discussed in section 4.4.2.

The lack of a training effect in Study Three adds to a substantial body of recent literature that report similar null effects of training tasks on indices of attention bias modification, which may represent manipulation failures, rather than evidence against any potential value of ABMT (e.g. Carlbring et al, 2012; Boettcher et al, 2012; Boettcher et al, 2013; Neubauer et al, 2013). As observed by Clarke et al (2014) failing to modify both selective attention and emotional vulnerability provides reassurance that the theoretical basis for ABM is sound (when combined with evidence that successful bias modification results in modified emotional vulnerability (see section 4.1.1).

4.4.2 Modification of attentional bias

A number of studies using ABMT utilising different methodologies and including various samples (healthy volunteers, highly trait and clinically anxious), yielded various results. A number of studies successfully using ABMT with healthy volunteers (e.g. MacLeod et al, 2002; Browning et al, 2010) have been criticised due to methodological problems (e.g. Hakamata et al, 2010), by including “attend threat” conditions and lacking a baseline bias assessment thus making evidence base of successful healthy volunteer studies limited. The ABMT task used in Study Three followed the task used by Hayes et al (2010), which reported successfully training those in the ABMT condition to faster identify targets in nonthreat than threat locations. Moreover those in the ABMT condition (as compared to control) subsequently reported fewer negative thought intrusions in a worry test, suggesting both a trained bias and an effect on emotion processing.

Importantly participants in Hayes et al (2010) sample were recruited on the basis of being high worriers, with required Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990) scores of 56 or more (one standard deviation below the mean for individuals diagnosed with GAD, Molina & Borkovec, 1994). Despite Hayes et al (2010) not including a manipulation check (i.e. no baseline bias was collected), it is reasonable to assume that this sample might have had a pre-existing threat bias. This is a very different sample than that used in Study Three, which consisted of healthy volunteers, who were rigorously screened prior to the session for mental health issues including anxiety. It is possible that the healthy volunteers were ‘too healthy’, and the screening excluded participants who naturally have a bias to threat and consequently are likely to benefit/respond to training away from threat. Consequently the lack of a training effect in Study Three might reflect a floor-effect (there was no vigilance to train into avoidance).

Another study using ABMT in highly trait anxious participants was Bar-Haim et al (2011), who used emotional-spatial cueing task with face stimuli (angry and a neutral expressions). Unlike Hayes et al (2010) who used a single study session, Bar-Haim (2011) used four treatment sessions. Stimuli display (top-to-bottom vs side-by-side), and number of training trials within a session (480 training trials divided into 10 blocks vs 384 trials divided into 8 blocks) also differed between Hayes et al (2010) and Bar-Haim et al (2011) respectively. Bar-Haim’s et al (2011) ABMT version included valid and invalid trials (where the probe appeared on the opposite location than the face), with 100% of the angry-face trials being invalid and 25% of neutral-face trials being invalid, providing a specific training bias to disengage attention from angry faces. In both studies the ABMT procedure was designed to train attention towards neutral stimuli/away from threat and in both the training led to differences in the bias between the active and control groups (although a

change in bias can only be confirmed in Bar-Haim's et al (2011) study). Both procedures also resulted in reduced vulnerability to stress in the subsequent behavioural tasks.

In studies with clinical samples Amir, et al (2009b) used word stimuli in a top-to-bottom design over eight sessions (240 trials per session) in sample of GAD patients training attention towards neutral stimuli/away from threat. Similarly to Bar-Haim et al (2011) the ABMT condition included some trials comprising of neutral words only and some mixed trials (probe always replacing the neutral word). Baseline bias was measured with no group differences. Post training the ABMT group showed significantly lower bias scores than the control group indicating a successful bias modification. Moreover 50% of participants in the ABMT group compared to 13% in control group no longer met diagnostic criteria for GAD after training. In a sample of SAD patients Amir et al (2009a) also over 8 sessions (160 trials per session) used stimuli comprising of disgust and neutral faces (Matsumoto & Ekman, 1989) in a top-to-bottom display (neutral + disgust, or neutral + neutral), again using mixed emotion and neutral only faces. Modified Posner task with eight social threat words (e.g. embarrassed, stupid, humiliated), and eight neutral words (e.g. dishwasher, tile, hanger) was used to assess bias at baseline and post-training. Groups did not differ in their baseline bias, a modified attentional bias was observed post treatment in the ABMT group, coupled with significant decreases in social anxiety (50% vs 14% no longer met diagnostic criteria in ABMT and control groups respectively). These clinical studies utilising various ABMT tasks report successful bias modification and changed emotional vulnerability. Importantly, both studies used repeated sessions and included some neutral only trials.

By contrast Boettcher et al (2013) were not successful in modifying selective attention in participants with SAD randomised into three groups: "towards positive", "towards negative" and a control condition. A modified dot probe task was used, and delivered via the internet in a 14-day programme with daily sessions, comprising of 192 trials, with paired stimuli consisting of either two differentially valenced words or pictures of facial expressions (happy, disgust, neutral). In one-third of the trials in each session stimulus pair members were neutral-negative, in one-third they were positive-negative, and in another they were neutral-positive. Importantly no attentional bias at baseline was present across groups and no change in attention bias was detected across conditions. However, symptoms of social anxiety reduced significantly from pre- to follow-up-assessment in all three conditions with the "towards threat" condition producing a significantly greater reduction of social fears relative to the control condition (with no significant differences between the "towards positive" and control condition). Intriguingly, changes in symptoms are unlikely to be via the mechanism of change in attention processes since there was no detected bias change.

Interestingly, all reported unsuccessful studies (e.g. Carlbring et al, 2012, Boechter et al 2012, 2013) conducted the training online – participants were allowed to self-administer the training from their own homes rather than having to attend lab sessions. This may be part of the problem – the task is long (20 min) and repetitive and thus motivation to complete it well may be low. Though the ABMT used in Study Three has been successfully used to train attention (Hayes et al, 2010), and was completed under controlled testing environments (i.e. in a test lab), it was still possible for participants to vary in task engagement and training.

In sum, ABMT studies use various methodologies and report varying results. As methodological differences between Study Three and other published studies exist, perhaps the failure to train attention might be attributed to one or a combination of those differences (e.g. type of stimuli, length of training session, sample characteristics). The meta-analysis by Hakamata et al (2010) revealed that ABMT protocols yield significantly larger effect sizes in clinical than healthy populations and that the number of sessions significantly predicts the effect of ABMT on attention bias – with larger amount of sessions increasing the effect. The majority of clinical (and high trait anxiety) studies utilised repeated administration protocols (e.g. 8 sessions Amir et al 2009a, 2009b; 4 sessions Bar-Haim et al, 2011). In contrast Study Three used a single session protocol as well as a healthy sample. As the clinical studies report an overall change in bias (pre-to post-treatment) but do not report any bias change assessment after a single session, it is difficult to assess whether a single session of ABMT can induce a bias change in highly anxious populations. Hayes et al (2010) utilised a single session protocol and reported a trained bias in an anxious sample, however no baseline bias assessment was performed. Importantly Study Three reported no existing baseline bias. Similarly, Boechter et al (2013) who also reported no baseline bias (although in a SAD sample) also failed to train bias. On the other hand studies that report a baseline bias (e.g. Amir et al 2009a, 2009b) report a successful modification of that bias (in clinical samples).

Hakamata et al (2010) suggests that studies using verbal target stimuli (words) generate a significantly larger effect than those using face stimuli. Moreover, studies using target stimuli presented in a top-bottom formation yield a significant effect size, while those presented in a side-by-side formation did not. Taken together these results suggest that ABMT consisting of word stimuli presented top-to-bottom is most effective in clinical and highly anxious populations, in repeated session protocols. With this in mind, Study Three used the more effective ABMT type (word stimuli), in the more effective display type, but this was used in a single session protocol, and more importantly in a healthy sample with no existing baseline attentional bias, suggesting that the failure to modify bias is largely due to the sample characteristics (floor effects).

4.4.3 Implications for future ABMT studies

ABMT studies should include reliable measures of bias at baseline/post-training. Study Three used a short measure of bias that was embedded in training blocks - this had the advantage of not diluting the training trials/effect but had the drawback of calculating bias scores from a small number of trials, which might in turn reduce the sensitivity/reliability of the bias score measure in the study (i.e. it is possible that the bias has been trained, but not measured adequately).

Moreover the visual probe task as a measure of selective attention has recently been criticised (e.g. Cooper et al, 2011; Kappenman et al, 2014) suggesting that it is a poor measure of attention (due to problems with reliability), and consequently is not best placed to measure changes in attention. Alternative tasks could be considered for use in attention training (e.g. Notebaert, Clarke, Grafton & MacLeod, 2014, developed a new person-identity-matching (PIM) task, with virtual cards, each depicting a happy and angry person and requiring to make matching judgements between two cards, and encouraging selective attention toward or away from threat, with success in both modifying attentional bias, and exerting an impact on emotional reactivity).

The Hakamata et al (2010) meta-analysis and the clinical anxiety studies (e.g. Amir et al 2009a, 2009b) suggest that repeated sessions should be used in order to elicit a meaningful change in attentional bias. The Boechter et al (2013) study which used the “train towards threat” condition provides interesting suggestion that despite this not modifying the attentional bias it may produce improvements in anxiety symptoms (perhaps in a mode similar to fear exposure in phobic patients). However, including a “train towards threat” condition rather than “train towards neutral” condition may have ethical considerations especially when used in a clinical sample.

Lastly, considerations should be made regarding sample selection. Hakamata et al (2010) report larger effect sizes of ABMT in clinical than non-clinical populations. When using a sample of healthy volunteers one cannot expect there to be a large baseline threat bias to be modified in the first place and thus such interventions may be better placed for clinical populations. In Study 3, the baseline threat bias was minimal in the group randomly assigned to receive the active training (1.86) and the group which was assigned the control condition had a slight bias towards neutral stimuli (-1.91). Perhaps ABMT should only be explored in samples in which a bias is likely to be observed (and can consequently be changed) as it is more likely to be able to alter an existing bias (present in clinical studies) than it is able to induce a non-existent bias (in non-clinical samples).

As the groups differed in their baseline attentional control (on ACS) perhaps the screening procedure could be altered in order to capture differences in attention control prior to the

experimental session. This could be achieved by administering the ACS during pre-session screening and randomising participants to the active and control ABMT groups on the basis of their ACS scores – with equal numbers of high and low ACS scoring participants per group.

Lastly, in Study Three the training was not administered during a state of increased anxiety – i.e. ABMT was performed prior to the 7.5% CO₂ challenge. It could be interesting to use the CO₂ model to induce anxiety during the ABMT training (simultaneous inhalation and training) but use a different stressful task used in other studies (e.g. a speech or an anagram task) post training. Attempting to modify attention in a highly anxious sample via the CO₂ challenge, could provide interesting results, potentially more similar to those in clinical samples, furthermore further validating the 7.5% CO₂ model of generalised anxiety.

v. Chapter 5

EEG brain activity during 7.5% Carbon Dioxide Inhalation in healthy humans.

5.1 Introduction

While the neuroimaging literature on anxiety has been largely focused on functional imaging methods, the limitations of fMRI such as poor temporal resolution, do not allow researchers to easily measure brain activity in real time (e.g. Crosson et al, 2010). Electroencephalography (EEG) can be a useful alternative, with the advantage of greater temporal resolution over fMRI to allow the study “on-line” brain activity across the cortex in real time.

5.1.1 Alpha band in anxiety

Investigations of regional brain activity using EEG in anxiety have observed increased levels of power in the Alpha band (8-12 Hz) in frontal regions, as well as a confirmation of the frontal asymmetry (see Chapter 1, section 1.8) in alpha activation (e.g. Schmidt, 1999; Beaton et al., 2008; Davidson et al., 2000).

This pattern of resting frontal EEG alpha asymmetry characterises depressed and anxious populations even when their symptoms are in remission (for reviews see Davidson 1993, 2000; Davidson et al 2003; Coan and Allen 2004). Clinical studies of social anxiety disorder (Davidson et al., 2000) as well as studies in non-clinical samples of adults selected for high levels of shyness (Schmidt, 1999) and social anxiety (Beaton et al., 2008) reveal greater relative right frontal EEG activity during both rest and periods of acute anxiety provocation. Moreover, healthy adults and children who exhibit greater relative right frontal EEG activity at rest report higher levels of distress, fear, and shyness, whereas those who exhibit greater relative left frontal EEG at rest are socially outgoing and extraverted (e.g. Sutton & Davidson 1997; Schmidt 1999; Fox 1991; Fox et al 2001).

Studies suggest that neurofeedback (biofeedback) training that aims to increase activation in specific networks (i.e. to counter existing asymmetry) can reduce baseline frontal asymmetry found in anxious individuals (e.g. Baehr, Rosenfeld & Baehr, 1997; Hammond, 2005; Choi et al, 2011; Peeters et al, 2014). For example three randomised active control studies in phobic anxiety (test anxiety), demonstrated that alpha EEG enhancement training produced 33% more alpha post-treatment, and significant reductions in test anxiety, compared to wait-list and relaxation control groups (Garrett & Silver, 1976).

Moscovitch et al (2011) measured the regional EEG activity at rest in 23 patients with social anxiety disorder (SAD) before and after CBT treatment and found that patients shifted significantly from greater right to greater left resting frontal brain activity from pre- to post-treatment. Moreover, greater left frontal EEG activity at pre-treatment predicted greater reduction in social anxiety from pre- to post-treatment and lower levels of post-treatment social anxiety.

Interestingly frontal EEG asymmetry in the alpha frequency band has been tentatively linked to attentional biases and proposed as one of the strongest biological correlates of 'affective style' (Davidson, 1995). Davidson (1995) proposed that for some individuals, stimuli that are perceived to be novel or ambiguous are categorized as threatening and trigger a withdrawal response (avoidant attentional bias) but for others the initial bias is marked by curiosity and an approach motivation (e.g., actively making new acquaintances). Withdrawal is linked to greater right frontal EEG activity at rest and in response to emotional provocation (Coan & Allen, 2004a; Harmon-Jones, Gable, & Peterson, 2010). This bias is evident in healthy children and adults (Coan, Allen, & Harmon-Jones, 2001; Fox, 1991), individuals at increased temperament- or trait-linked risk for anxiety and depression (Fox et al., 1995; Schmidt, 1999), and individuals with a current or past mood disorder (Allen & Kline, 2004; Kentgen et al., 2000). In contrast, greater left frontal EEG activity has been linked to approach tendencies, involving both positive emotions, such as joy (Ekman & Davidson, 1993), and negative emotions, such as anger (Harmon-Jones, 2007).

5.1.2 Unique role of frontal regions?

Recently Pérez-Edgar et al (2013) observed frontal EEG asymmetry patterns at rest and under social threat (a stressful speech condition) among young adults. Frontal EEG asymmetry at baseline did not predict attention bias patterns to angry or happy faces. However, increases in right frontal alpha asymmetry from baseline to the stressful speech condition were associated with vigilance to angry faces and avoidance of happy faces. These findings may reflect individual differences in the pattern of response (approach or withdrawal) to a mild stressor. Further analyses of frontal beta asymmetry and parietal alpha asymmetry did not find similar patterns. Consequently these data may reflect the unique role of alpha activity in frontal regions, particularly the DLPFC, in cognitive control and threat detection.

In an earlier study Heller et al (1997) studied power in the alpha band in frontal and parietal areas in a large group of non-clinical participants during periods of rest and during an emotional narrative task designed to elicit anxiety. Their results showed that participants who previously scored high in measures of trait anxiety (measured using the trait version of the State Trait

Anxiety Inventory STAI; Spielberger et al 1983), showed an overall increase in alpha power in left frontal sites compared to low anxious participants. Furthermore, increased alpha band power was also seen in parietal sites in the right hemisphere when trait anxious participants listened to sad or fearful narratives which evoked increased levels of anxious arousal (e.g. increased heart rate/blood pressure) again compared to low anxious controls.

Similar results have been observed in anxious patients when compared to healthy controls. For example, Mathersul, Williams, Hopkinson and Kemp (2008) reported an overall increase in right frontal alpha band power in their clinical sample of anxiety patients who reported negative mood. Further still, greater alpha power in right frontal sites was associated with anxious arousal across clinical and healthy participants whereas more alpha power in left frontal and left parietotemporal sites was associated with anxious apprehension (e.g. worry/tension).

5.1.3 Other frequency bands

Gamma

Recent research suggests a relationship between EEG gamma activity and negative emotional processing (e.g. Luo et al., 2007; Matsumoto et al., 2006). Intracranial field potentials recorded from the amygdala confirm that gamma power is highest for aversive stimulus presentations as compared with neutral or pleasant stimuli (Oya et al., 2002). When asked to imagine a phobic object, individuals suffering from a specific phobia show increases in gamma band activation as well as increases in heart rate and respiration (Gemignani et al., 2000). Also, gamma activity has been shown to decrease during periods of relaxation and to increase during periods of imagining negative emotional material (Sebastiani et al., 2003).

Oathes et al (2008) examined EEG gamma (35–70 Hz) spectral power distributions during worry induction task in participants with GAD and in healthy controls with no history of psychiatric illness. They found that the EEG gamma band could differentiate worry from baseline and relaxation. During worry induction, GAD patients showed higher levels of gamma activity than control participants in posterior electrode sites that have been previously associated with negative emotion. Moreover the gamma fluctuations in these electrode sites were correlated with subjective emotional experience ratings lending additional support to interpretations of negative affect. The GAD patients next underwent 14 weeks of psychotherapy before their gamma activity was measured again during a worry task. Post treatment the GAD group reported less negative affect during the worry induction task and their gamma activity no longer differed from control participants. These findings suggest converging evidence that patients suffering from

GAD experience more negative emotion during worry and that the EEG gamma band is useful for monitoring fluctuations in pathological worry and treatment response.

Oathes et al (2008) found the presence of the gamma band was significantly higher in their sample of GAD patients who were highly anxious after engaging in a worry induction task compared to a second group of normal controls. Moreover, Oathes and colleagues also reported that gamma activity was asymmetric in GAD patients, with an overall increase in gamma activity appearing in the left temporal area during worry induction.

Theta

Recently Dadashi et al (2015) used a quasi-experimental study to examine the effects of neurofeedback on theta activity in GAD. Patients in both groups were evaluated at pre-test and post-test with Generalised Anxiety Disorder Scale (GAD-7) and Global Assessment Functioning Scale (GAFs). The treatment group received fifteen 30-minute neurofeedback alpha training sessions and fifteen 30-minute theta brain training sessions. The results showed that increase of alpha and theta brain waves amplitude in occipital area in people with GAD can increase the global functioning level and reduce symptoms of GAD in the treatment group, relative to the waiting list group. After the completion of the study the waiting list group continued to fulfil diagnostic criteria for GAD whilst the treatment group did not. As the training increased both alpha and theta, it is difficult to assign a causal effect of the theta or the alpha band separately. A review by Moore (2000) indicated that both: alpha and theta as well as alpha-theta enhancements are effective treatments of the anxiety disorders including GAD.

There are differences in activation of right and left prefrontal cortex between healthy individuals and those with affective and anxiety disorders (Davidson, 1998a). Davidson and Fox proposed that the frontal lobes are differentially involved in positive versus negative affective states and corresponding behaviours (Davidson, 2000; Fox, 1991), with left frontal areas mediating the experience of positive emotions and approach behaviours, and right frontal areas mediating the experience of negative emotions and withdrawal behaviours. A large number of EEG studies (e.g. Davidson, 1992, 1995, 1998a) provide support for this frontal asymmetry.

In sum, the reviewed literature suggests that anxious individuals have different brain activation at rest and during anxious arousal compared to healthy controls, characterised by elevations in right frontal alpha and posterior gamma, but overall decreases in theta.

The effects of CO₂ challenge on subjective, autonomic and neuropsychological characteristics of anxiety are well known. The current study tested whether CO₂ challenge (vs. air) might induce patterns of EEG activity that characterise anxious populations. The primary analysis focused on

the alpha band, specifically differences in activation due to frontality and laterality, expecting increased alpha in the right frontal cortex. An exploratory analysis of frontal versus occipital sites was performed following the report from Dadashi et al (2015) of an increase in alpha and theta activation there due to biofeedback training.

HA = 20 minutes of the 7.5% CO₂ challenge in healthy humans will produce a pattern of brain activation (alpha, gamma and theta frequency bands) different to that observed in healthy humans at rest (20 minutes of air inhalation).

HA2 = 20 minutes of the 7.5% CO₂ challenge in healthy humans will result in more alpha power in the right (vs left) frontal cortex.

5.2 Method

5.2.1 Design

A within-subjects design compared healthy participant brain activation at rest during 20 minutes of normal air inhalation and during 20 minutes of the 7.5% CO₂ challenge (gas order counterbalanced within male and female participants). Post-inhalation measures of subjective anxiety and autonomic arousal were taken at the end of both inhalations.

5.2.2 Participants

Twenty five eligible participants were recruited (12 females, mean age of 22.16 SD = 2.63). The screening procedure and exclusion criteria were identical to those described in Chapter 2 (section 2.2). In brief, all participants were healthy volunteers required to be aged 18-55 years, who underwent a telephone health screen prior to the testing session. On the day of the study session participants underwent a further physical and mental health screen using a pre-test diagnostic interview (Mini International Neuropsychiatric Interview MINI) (Sheehan et al., 1998).

Exclusion criteria for this study were: history of epilepsy, history of mental health illness including anxiety and depression, high blood pressure (>140/90), use of medication in the past 8 weeks prior to testing other than local treatments, paracetamol/aspirin or contraceptives, a Body Mass Index (BMI) of < 18, or >28, and history of respiratory or cardiovascular problems including asthma. Participants' alcohol intake was required to be no greater than 50 units per week (for males) and 35 units per week (for females).

Recruitment and screening procedures and test protocols were approved by the Research Ethics and Governance Committee at the University of Southampton. Study Information, Consent and Debrief forms are presented in Appendices (A5; A13-A14).

A power calculation was performed *a priori* for a repeated measures within-factors ANOVA with 3 measurements (band frequency x frontality x laterality) which revealed that the sample size needed to detect a modest effect size (Cohen's $d = 0.6$) with the alpha level of .05 and high power of 0.8 was $n=25$. An effect size of approximately this magnitude was expected, informed by the results of previous within-subjects EEG studies (e.g. in Pérez-Edgar et al (2013) effect size for an alpha asymmetry valence x time interaction was , $d=0.78$).

5.2.3 Materials

This study used the same measures as those reported in Chapter 2 (section 2.2). In brief, these included measures of trait anxiety (STAI, GAD-7, PSWQ, ASI), attention control (ACS), state mood (PANAS, SSAI, VAS) and heart rate and blood pressure. Please see Appendix A6 for scales.

EEG data acquisition & signal processing

EEG data was continually recorded from 30 equidistant Ag/AgCl electrode sites (Fp1, Fp2, F7, F3, Fz, F4, F8, Ft7, Fc3, Fcz, Fc4, Ft8, T7, C3, Cz, C4, C4, T8, Tp7, Cp3, Cpz, Cp4, Tp8, P7, P3, Pz, P4, P8, O1, Oz and O2) placed in accordance with the extended 10/20 system using an electrode cap (Easycap; Hersching, Germany) (see Figure 9). All scalp electrodes were referenced to the nose (e.g. Kaganovich, Francis & Melara, 2006; Manna et al, 2010). Afz (a site in front of Fz) was used as the ground (e.g. Suway et al, 2013; Jaworska et al, 2012).

Data were gathered using a Neuroscan Synamps2 70 channel EEG system (version 4.5). Vertical eye movements (to correct for artefact rejection) were recorded using two electro-oculogram (EOG) electrodes which were placed above and below the right eye. Head movements were also recorded using further two electrodes which were placed on the left and right temporal area of the head. Impedance was kept below 5 k Ω for all electrodes.

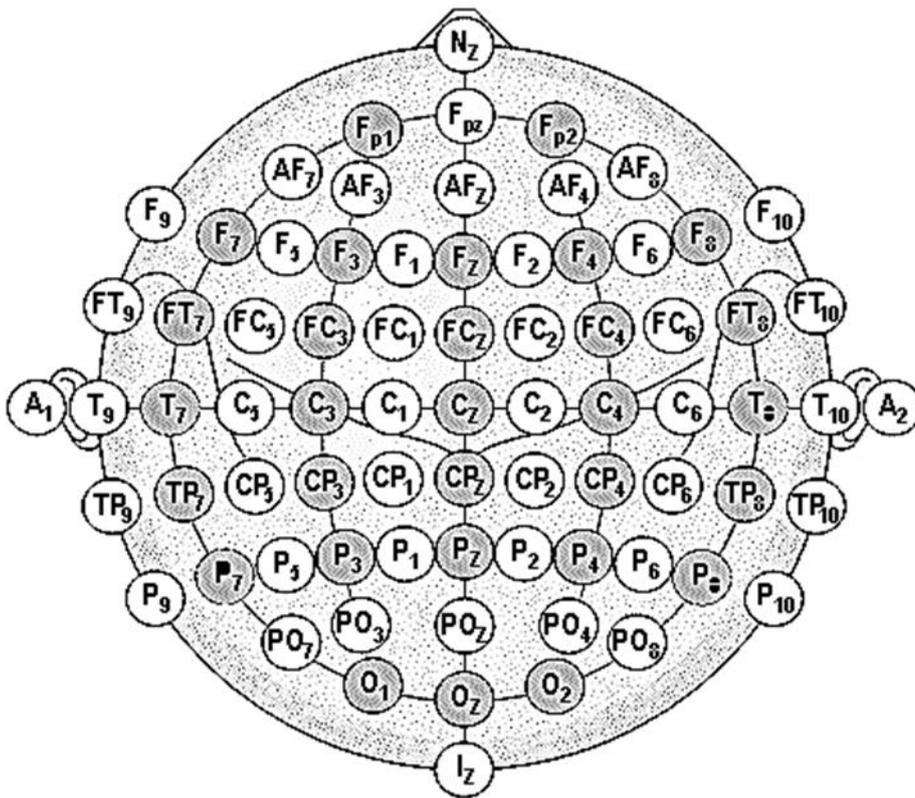


Figure 9 The extended 10/20 EEG system.

5.2.4 Procedure

Consistent with previous chapters participants responded to a study advert and were telephone screened. Eligible participants were instructed to refrain from alcohol and caffeine consumption for 24 hours prior to the test session. On the study day participants provided informed consent and underwent further screening with the MINI and blood pressure/heart rate. Testing was completed in testing lab in the Academic Unit of Psychology with controlled lighting and temperature. Eligible participants completed trait measures (STAI, ASI, GAD-7, ACS, PSWQ) and baseline state measures of anxiety and mood (SSAI, PANAS, GAD-7 state and VAS).

Baseline measures of blood pressure and heart rate were taken and participants were then fitted with the 32-channel EEG cap and EOG electrodes. Participants completed two 20 minute inhalation sessions of 7.5% CO₂ enriched air (21% O₂; balance N₂) and normal/medical air. Administration of the gas through an oro-nasal face mask (see Figure 15, Appendix A3) and the gas order was single blind. Measures of autonomic arousal as well as measures of state mood/anxiety were recorded both immediately after each inhalation period. The test session per participant lasted approximately 3 hours.

Data Processing

Offline signal processing was carried out following Finnigan & Robertson (2011) with Edit 4.4 (Compumedics-Neuroscan USA Ltd). Data files were each separated into contiguous epochs of 1024 data points (2 s). Epochs in which the EEG amplitude exceeded $\pm 80 \mu\text{v}$ were rejected. From the first 90 epochs of artefact free data per participant, EEG power was computed for each electrode and each 0.5 Hz bin using fast Fourier Transform (FFT) with a cosine window (length: 10%). Absolute power for each electrode was summed across the delta (2-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-25 Hz) & gamma (35-70 Hz) bands. Relative power for each band was computed as the ratio of absolute band power to total power across the 0.5-70 Hz range.

5.3 Results

5.3.1 Group Characteristics

Data from 25 participants were collected. In order to detect any possible effects of gas order on either the brain activation, mood, self-report anxiety measures or autonomic arousal, gas order has been used as a between groups factor. Independent samples t-tests indicate that there were no baseline differences on any of the measures between the individuals who underwent air inhalation first, followed by CO₂, and those who underwent CO₂ inhalation first, followed by air (See Table 23, Appendix A16).

For group characteristics at baseline (collapsed across groups) see Table 20.

Table 20

Group Characteristics (All Participants)

	Baseline	
	M	SD
GAD-7	23.66	16.50
STAI	34.38	6.75
ACS	54.53	8.97
ASI	56.00	30.94
PSWQ	39.11	11.32
PANAS positive affect	33.71	6.31
PANAS negative affect	13.83	3.96
VAS negative affect	26.58	21.21
VAS positive affect	110.93	15.95
VAS cognition	77.21	43.04
HR	74.52	10.05
SBP	121.04	12.94
DBP	71.76	7.95

STAI= Spielberger Trait Anxiety Inventory; ACS = Attention Control Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire; Positive and Negative Affect Scale; HR= Heart rate; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure.

5.3.2 Effects of CO₂ on mood and subjective anxiety

As expected there was a large effect of 7.5% CO₂ inhalation on self-reported anxiety and mood measures. Separate mixed design analyses of variance (ANOVA) (2 x 3) with a between subjects factor of gas order (CO₂ first vs air first) and a within subjects factor of time (baseline, post-air and post-CO₂) were conducted for measures of GAD7, STAI, VAS cognition, VAS positive affect, VAS negative affect, PANAS negative affect and PANAS positive affect. A significant main effect of time was revealed for all but one (VAS cognition ($F(2,23)=1.14, p=.33$)) of the self-report anxiety and

mood measures ($F's > 17.03$, $p's < .001$, $\eta^2 P's > 0.44$). There were no main effects of gas order or gas-order x time interactions for any of the measures ($F's < 2.69$, $p's > .08^{27}$).

Paired comparisons between each time point revealed significantly higher anxiety and negative affect ratings, and significantly lower positive affect, following CO₂-challenge (relative to baseline and post-air inhalation), but no differences between baseline and post-air inhalation for GAD7, PANAS negative affect, and VAS negative affect, ($p's < .05$, see superscripts in Table 21). For VAS positive affect and PANAS positive affect, there were significant decreases in positive affect post air-inhalation relative to baseline; and even larger decreases post CO₂-inhalation. State anxiety increased significantly following the air-inhalation relative to baseline, with no significant difference between air and CO₂ inhalations. There were no statistically significant changes in VAS cognition following either of the inhalations (see superscripts in Table 21).

These results suggest that 20 minutes of 7.5% CO₂ challenge lead to significant decreases in positive affect as well as significant increases in negative affect and self-reported anxiety, and these anxiolytic effects are significantly larger than those following normal air inhalation, validating this experimental model.

Means and standard deviation values are presented in table 21.

²⁷ This trend was for time x gas order interaction for State Anxiety, characterised by higher reported state anxiety ratings after the air inhalation in the group receiving air first, as compared to the group receiving it second ($m = 45.91$ vs $m = 39.46$ in air first and air second respectively). Anxiety ratings at baseline and post CO₂ inhalation were comparable. All other gas order effects and interactions were non-significant with $F's < 1.62$, $p's > .22$).

Table 21

The Effect of 7.5% CO₂ Inhalation on Mood and Subjective Anxiety

	Baseline		Air		7.5% CO ₂		ANOVA
	M	SD	M	SD	M	SD	F(2,24) =
GAD-7	23.66 ^a	16.50	22.70 ^a	23.65	67.97 ^b	40.24	27.83, p<.001
State Anxiety	34.38 ^a	6.75	42.42 ^b	9.96	44.96 ^b	9.23	17.03, p<.001
PANAS positive affect	33.71 ^a	6.31	25.58 ^b	7.63	23.83 ^c	7.65	27.29, p<.001
PANAS negative affect	13.83 ^a	3.96	12.92 ^a	4.19	22.21 ^b	10.03	18.86, p<.001
VAS negative affect	26.58 ^a	21.21	20.63 ^a	20.48	71.50 ^b	48.54	20.78, p<.001
VAS positive affect	110.93 ^a	15.95	91.27 ^b	23.13	45.27 ^c	32.77	48.16, p<.001
VAS cognition	77.21 ^a	43.04	63.08 ^a	44.99	73.83 ^a	47.80	1.14, p=.33

Baseline measure is Trait Anxiety (Spielberger Trait Anxiety Inventory), post-air and post CO₂ the state measure is used (Spielberger State Anxiety Inventory); PANAS = Positive and Negative Affect Scale; VAS = Visual Analogue Scale

Within each measure values with different superscripts significantly differ, $p < .05$

5.3.3 Effects of CO₂ on autonomic arousal

As expected there was a strong effect of 7.5% CO₂ inhalation on autonomic arousal. A mixed design analyses of variance (ANOVA) (2 x 3) for heart rate, systolic and diastolic blood pressure revealed a significant main effect of time for HR ($F(2,23)=53.58$, $p<.001$, $\eta^2P=0.70$) and SBP ($F(2,23)=27.34$, $p<.001$, $\eta^2P=0.54$). The effect of time did not reach significance for DBP ($F(2,23)=2.69$, $p=.08$, $\eta^2P=0.11$). There were no main effects of gas order or gas-order x time interactions for any of the autonomic arousal measures ($F's<3.02$, $p's>.10$).

Paired comparisons between each time point revealed significantly lower HR following the air-inhalation (relative to baseline and CO₂-inhalation) and significantly higher HR following the 7.5% CO₂ challenge as compared to baseline and air-inhalation ($p's < .05$, see superscripts in Table 22). SBP was significantly higher following the CO₂ inhalation as compared to baseline and air-inhalation, and SBP was significantly higher post CO₂-inhalation as compared to air-inhalation only ($p's < .05$, see superscripts in Table 22).

These results suggest that 20 minutes of 7.5% CO₂ challenge leads to significant increases in autonomic arousal.

Means and standard deviation values are presented in table 22.

Table 22

The Effect of 7.5% CO₂ Inhalation on Autonomic Arousal

	Baseline		Air		CO ₂		ANOVA F(2,24) =
	M	SD	M	SD	M	SD	
HR	74.52 ^a	10.05	70.56 ^b	9.88	93.08 ^c	16.20	53.58, $p<.001$
SBP	121.04 ^a	12.94	118.84 ^a	9.28	132.60 ^b	10.09	27.34, $p<.001$
DBP	71.76 ^{ab}	7.95	70.60 ^a	6.21	74.36 ^b	7.98	2.69, $p=.08$

SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure

5.3.4 Effects of 7.5% CO₂ on the EEG frequency bands' activity

The primary analysis focused on the alpha band, specifically differences in activation due to frontality and laterality and so the electrodes were grouped into: left frontal (FP1, F7, F3) and right frontal sites (FP2, F8, F4) (consistent with Allen, Coan & Nazarian, 2004), left parietal (TP7, CP3, P3, P7), and right parietal (CP4, TP8, P4, P8) sites and left occipital (O1) and right occipital (O2) sites (see Appendix A15 for a display of groupings).

Secondary analyses were conducted for the gamma and theta frequency bands. Consequently three 2 (left vs. right) x 3 (frontal vs. parietal vs. occipital) x 2 (CO₂ vs. air) repeated ANOVAs were conducted separately for measures of alpha, gamma and theta.

Data were screened for outliers prior to the main analyses. Data from two participants were excluded: one participant was an extreme outlier on overall mean alpha band activity during air inhalation (high score of mean_alpha_air = 46.99 (mean = 9.90, SD = 9.38)) as well as during the 7.5% CO₂ challenge (high score of mean_alpha_CO₂ = 17.59 (mean = 7.34, SD = 4.12)). A second participant was an extreme outlier on both mean gamma activity during normal air inhalation (13.75, mean = 1.42, SD = 2.67) and on theta activity during the 7.5% CO₂ challenge (30.08, mean = 14.90, SD = 7.14). Subsequently data from these two participants were excluded from the main analyses.

5.3.5 Effects of 7.5% CO₂ challenge on alpha band activation

A mixed design analyses of variance (ANOVA) revealed a significant main effect of frontality ($F(2,21) = 32.45, p < .001$), and a significant main effect of gas ($F(1,21) = 4.86, p < .05$). There was also a significant frontality x gas interaction ($F(2,21) = 6.64, p < .01$). No other significant main effects or interactions emerged ($F's < 1.61, p's > .21$). See Figure 10.

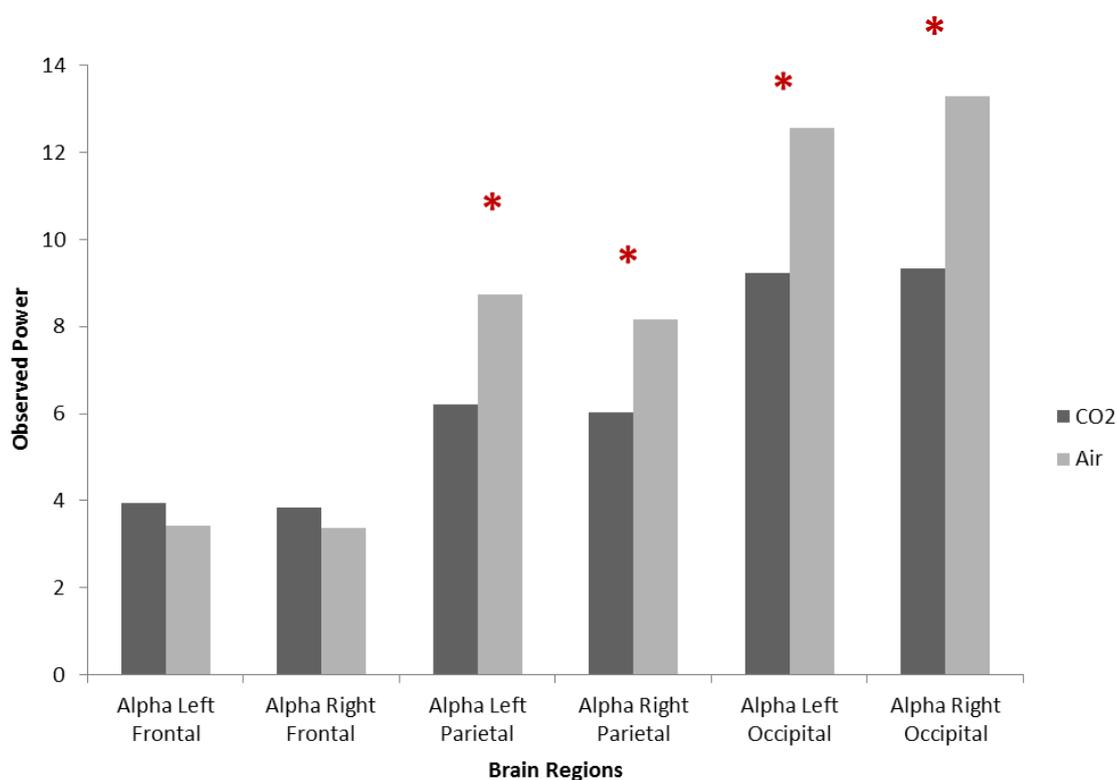
The significant main effect of frontality was characterised by an overall lower activity in the frontal region ($m = 3.66$) as compared to the parietal ($m = 7.51$) and occipital regions ($m = 10.03$). The significant main effect of gas was characterised by overall decreases in alpha band activation following the CO₂ inhalation ($m = 5.68$) as compared with air inhalation ($m = 8.45$).

In order to investigate the interaction separate 2-way ANOVAS (laterality x gas) were performed for frontal, parietal and occipital regions. For frontal regions there was a significant main effect of laterality ($F(2,21) = 5.09, p = .03, \eta^2 P = 0.19$), characterised by significantly higher alpha in left vs right sites (collapsed across the inhalations). No main effect of gas nor a gas x laterality interaction emerged ($F's < 1.37, p > .26$).

For parietal regions there was a main effect of gas ($F(2,21) = 7.50, p = .01, \eta^2 P = 0.25$), characterised by significantly lower alpha during CO₂ as compared with air-inhalation. No main effect of laterality and no laterality x gas interaction emerged ($F's < 1.36, p's > .26$).

For occipital regions similarly there was a main effect of gas ($F(2,21) = 5.11, p = 0.3, \eta^2 P = 0.19$), characterised by significantly lower alpha during the CO₂ as compared with air-inhalation. No main effect of laterality and no laterality x gas interaction emerged ($F's < 1.03, p's > .32$).

These findings suggest that twenty minutes of 7.5% CO₂ challenge result in significantly lower alpha activity in parietal and occipital regions, with this effect greatest at right occipital regions. In contrast there was some evidence that CO₂ increased alpha across left and right frontal sites.



*= $p < .05$

Figure 10 Differences between normal air inhalation and the 7.5% CO₂ challenge on alpha band activation across cortical sites.

5.3.6 Effects of 7.5% CO₂ on gamma band activation

A mixed design analyses of variance (ANOVA) (2 x 3) revealed a significant main effect of gas ($F(1,21)=9.31$, $p=.01$, $\eta^2P=0.30$), and a significant gas x frontality interaction ($F(2,21)=3.74$, $p=.03$, $\eta^2P=0.15$). No other significant main effects or interactions were revealed (F 's < 2.76, p 's > .07²⁸) (see Figure 11).

The significant main effect of gas was characterised by overall higher gamma band activation following the CO₂ inhalation ($m = 2.57$) as compared to air inhalation ($m = 0.87$).

²⁸ This was a trend for an effect of frontality, characterised by significantly lower gamma (collapsed across inhalations) in parietal relative to occipital and frontal regions (with no meaningful differences between occipital and frontal regions). Other non-significant main effects and interactions were ($F < 0.73$, p 's > .40).

The significant gas x frontality interaction was investigated further by performing separate analyses for CO₂ and air-inhalations (frontality x laterality). For air there were no significant main effects or interactions (F 's<0.86, p >.42). For CO₂ there was a significant main effect of frontality ($F(2,21)=5.38$, $p<.01$, $\eta^2P=0.20$), characterised by significantly higher gamma in occipital regions compared to frontal regions ($p=.04$) and parietal regions ($p=.01$) (with no meaningful difference between frontal and parietal regions).

These findings suggest that twenty minutes of 7.5% CO₂ challenge result in significantly higher gamma activity across all sites, with this effect greatest at occipital regions. In contrast the gamma elevation was least prominent in left frontal regions.

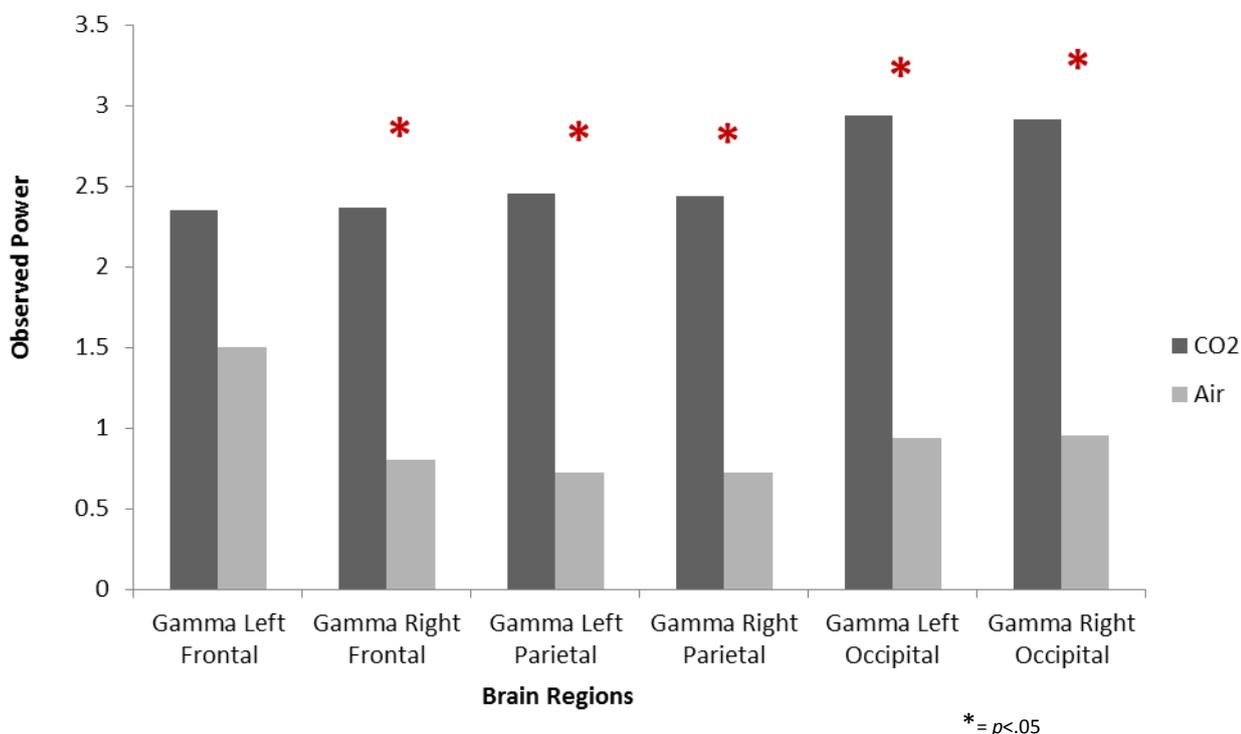


Figure 11 Differences between normal air inhalation and the 7.5% CO₂ challenge on gamma band activation.

5.3.7 Effects of 7.5% CO₂ on theta band activation

A mixed design analyses of variance (ANOVA) (2 x 3) for the theta frequency band activation revealed a significant main effect of gas ($F(1,21) = 22.39$, $p<.001$, $\eta^2P=0.50$), a significant main effect of frontality ($F(2,21) = 3.73$, $p<.01$, $\eta^2P=0.15$) as well as a significant frontality x gas

interaction ($F(2,21)=7.78$, $p<.001$, $\eta^2P=0.26$). No other significant main effects or interactions were revealed ($F's<3.44$, $p's>.08^{29}$).

The significant main effect of gas was characterised by overall increases in theta band activation following the CO₂ inhalation ($m = 14.19$) as compared to air inhalation ($m = 9.09$). The significant main effect of frontality was characterised by significantly lower theta (collapsed across inhalations) in the parietal relative to occipital and frontal regions, with no differences between occipital and frontal regions ($p's <.04$).

The significant gas x frontality interaction was investigated further by performing separate analyses for CO₂ and air-inhalations (frontality x laterality). For air there was a trend for a main effect of frontality ($F(2,21)=2.84$, $p=.07$, $\eta^2P=0.11$), characterised by lowest theta in parietal sites, (significantly lower relative to occipital regions, $p<.001$). No other main effects or interactions emerged ($F's<2.33$, $p>.14$). For CO₂ there was a significant main effect of frontality ($F(2,21)=7.54$, $p<.01$, $\eta^2P=0.26$), characterised by lowest theta in parietal regions (significantly lower relative to frontal ($p<.01$) and occipital regions ($p<.001$) (with no meaningful difference between frontal and occipital regions).

These findings suggest that twenty minutes of 7.5% CO₂ challenge result in significantly higher theta activity across all sites, with this effect greatest at frontal regions. In contrast the effect of CO₂ on theta was weakest at parietal sites (see Figure 12).

²⁹ This was a trend for the main effect of laterality, characterised by higher theta in left vs right regions (collapsed across frontality and gas). Other non-significant effects and interactions were ($F's<1.43$, $p's>.25$).

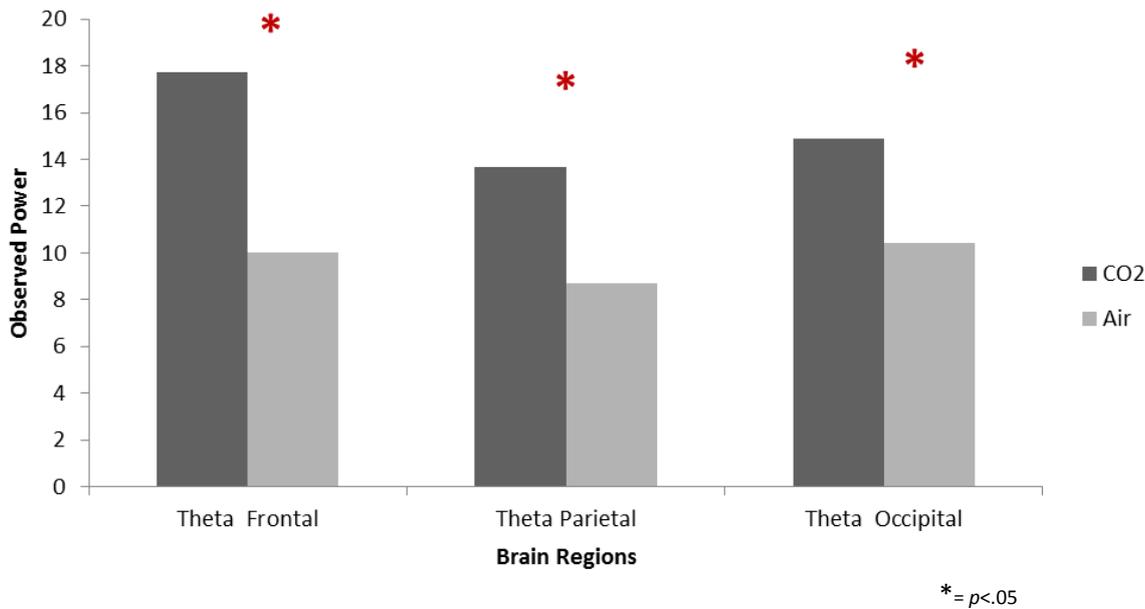


Figure 12 Differences between normal air inhalation and the 7.5% CO₂ challenge on theta band activation

5.3.8 Correlations

Pearson's correlations were performed in order to examine if any of the changes (delta scores) in alpha, gamma and theta activation between the CO₂ inhalation to air were associated with changes in autonomic arousal or subjective anxiety.

Regarding changes in autonomic arousal, the CO₂-induced decrease in alpha in left parietal cortex was significantly negatively correlated with the increase in SBP ($r = -0.42$, $p = .05$). There was a trend for an association between the increase in alpha in right and left frontal cortex and the increase in SBP post CO₂ ($r = 0.40$, $p = .06$; $r = 0.38$, $p = .08$ respectively). There were no other significant associations between changes in alpha and changes in autonomic arousal, and no significant associations between changes in gamma and theta and changes in CO₂-induced autonomic arousal (r 's < 0.32 ; p 's $> .14$).

Left and right parietal decreases in alpha were also significantly correlated with CO₂-induced increases in negative affect (measured on PANAS negative affect) ($r = -0.47$, $p = .02$; $r = -0.51$, $p = .01$, respectively). No significant associations emerged between changes in any other mood and subjective anxiety measures and alpha changes in other sites, nor between any of the measures and changes in gamma and theta (r 's < 0.35 , p 's $> .10$).

A number of significant associations between changes in subjective anxiety and changes in autonomic arousal were also found. These were positive correlations between changes in SSAI, GAD7, PANAS negative affect and VAS negative affect and changes in SBP (r 's > 0.46 , p 's $< .05$).

Moreover there were a number of significant correlations between post-CO₂ alpha and theta. Increases in left and right frontal alpha were correlated with increases in theta across all sites ($r's > 0.82$, $p's < .001$). Moreover significant associations emerged between post-CO₂ decreases in left parietal alpha and increases in left and right parietal and left and right occipital theta ($r's > -0.47$, $p's < .05$). There were trends for a significant association between decreases in left parietal alpha and increases in left and right frontal theta ($r's < -0.38$, $p's > .07$). No significant correlations emerged between post-CO₂ right parietal, left occipital and right occipital alpha and increases in theta ($r's < -0.35$, $p's > .10$). There were no significant correlations between any changes in alpha and gamma or between changes in gamma and theta ($r's < 0.29$, $p's > .18$).

5.4 Discussion

5.4.1 Changes in Mood and Autonomic Arousal

As expected 7.5% CO₂ inhalation increased self-reported anxiety and negative affect and decreased positive affect. Moreover there was a strong effect of 7.5% CO₂ inhalation on autonomic arousal characterised by increased heart rate and blood pressure following the CO₂ inhalation. These results were consistent with those reported in Chapter 3 and 4 and in previous studies (Bailey et al. 2005; Bailey and Nutt 2008; Bailey et al. 2011a; Poma et al. 2005).

5.4.2 Frequency bands differences

CO₂-challenge produced non-significant increases in frontal alpha activity, combined with large decreases in activity in parietal and occipital regions. Conversely CO₂ challenge increased gamma and theta activity across all measured cortical brain regions, this effect being largest in occipital regions for gamma, and frontal regions for theta. Increases in frontal alpha, decreases in posterior alpha and increases in gamma were predicted following prior evidence that highly trait and clinically anxious individuals show an overall increase in in both right and left frontal alpha band power (e.g. Heller et al, 1997; Mathersul et al, 2008), and that GAD patients display a significantly higher presence of the gamma band (particularly at parietal sites) than healthy controls (e.g. Oathes et al, 2008). However the overall observed increases in theta were contrary to study hypotheses and prior evidence that low rather than high theta activation is associated with anxiety (e.g. Lagopoulos et al, 2009; Demerdzieva, 2011).

Moreover, no correlations were observed between changes in gamma and theta activation and changes in subjective anxiety or autonomic arousal, whereas effects of CO₂ on alpha activity (at left parietal sites, and trend-wise at frontal sites) were associated with increases in SBP; and left

and right parietal decreases in alpha were also significantly correlated with CO₂-induced increases in negative affect (measured on PANAS). This suggests that anxiety is more strongly associated with changes in alpha activity, or that 7.5% CO₂ inhalation is especially able to induce changes in alpha.

5.4.3 Alpha

Contrary to the hypothesis, no main effect of laterality was observed in the alpha frequency band. When examining the three brain regions separately, there was an overall main effect of laterality in frontal cortex only (collapsed across the inhalations), characterised by overall higher alpha in left vs. right frontal sites. The significant main effect of frontality was characterised by an overall lower activity in the frontal region as compared to parietal and occipital regions (collapsed across air and CO₂ inhalations). The CO₂ challenge resulted in significantly lower alpha activation in parietal and occipital regions as compared to air inhalation. The increases in alpha activation in frontal regions following the CO₂ inhalation were not statistically significant as compared to air.

Significant differences were expected primarily between left and right frontal regions (frontal asymmetry hypothesis), and thus it was surprising to see that 7.5% CO₂ challenge resulted in increases in both left and right frontal alpha activity as compared to air (albeit not statistically significantly so). Current literature suggests that anxious individuals frequently display a frontal asymmetry, whereby at rest, they display higher alpha activation in right frontal regions as compared to left. For example Davidson and Fox proposed that the left frontal areas mediate the experience of positive emotions and approach behaviours, and right frontal areas mediate the experience of negative emotions and withdrawal behaviours (Davidson, 2000; Fox, 1991), with a large number of EEG studies supporting this view (e.g. Davidson, 1992, 1995, 1998a). More recently studies with clinical subjects diagnosed with SAD (Davidson et al., 2000) as well as studies with non-clinical samples of adults selected for high levels of shyness (Schmidt, 1999) and social anxiety (Beaton et al., 2008) have been shown to exhibit significant relative elevations in right frontal brain activity when assessed during resting states or periods of acute emotional provocation.

Recently Pérez-Edgar et al, (2013) found that increases in right frontal alpha asymmetry from baseline to a stressful speech condition were associated with vigilance to angry faces and avoidance of happy faces in an emotion-face dot-probe attention bias task. They concluded that this may reflect the unique role of frontal regions, particularly the DLPFC, in cognitive control and threat detection, coupled with ruminative processes associated with alpha activity.

However, some studies have also reported contrary findings. Mathersul, Williams, Hopkinson and Kemp (2008) reported an overall increase in right frontal alpha band power in patients with clinical anxiety, and in individuals demonstrating anxious arousal, but greater alpha power in left frontal and left parieto-temporal sites in those reporting worry and tension. A study by Heller et al (1997) further supports this view. Power in the alpha band in frontal and parietal sites was studied in a large group of non-clinical participants during periods of rest and during an anxiety-inducing task. Results indicated that high trait anxious participants showed an overall increase in alpha power in left frontal sites compared to low anxious participants. Moreover, to further support the distinction between the two aspects of anxiety (anxious arousal vs. anxious apprehension), studies have found that although worry increases reports of negative affect and anxiety, particular physiological systems may not register emotional arousal during worry. When asked to worry, research participants report increases in anxiety while cardiovascular measures do not consistently reflect the change (Borkovec & Hu, 1990; Borkovec et al, 1993).

Together these findings suggest that autonomic arousal may covary with right frontal alpha power and cognitive anxiety with increases in left frontal alpha power.

The frontality analyses findings seem to broadly correspond (e.g. Heller et al, 1997; Mathersul et al, 2008; Pérez-Edgar et al, 2013), but also contrast with some of the available literature on alpha frontality in anxiety. These reported studies differ in terms of laterality findings, but all report significant increases in alpha in frontal regions as compared to other sites. In the current study small increases in both left and right frontal alpha were observed relative to air inhalation (albeit not reaching statistical significance).

However in the Heller et al (1997) study further to the increased alpha band power in left frontal regions, increased alpha was seen also in parietal sites in the right hemisphere when these same highly trait anxious participants listened to sad or fearful narratives which evoked increased levels of anxious arousal e.g. increased heart rate/blood pressure compared to low anxious controls. This is a markedly different finding to the one presented in this 7.5% CO₂ study, where highly significant decreases rather than increases in parietal (and occipital) alpha were observed due to the anxiolytic effects of the 7.5% CO₂ inhalation. Even more interestingly, in this 7.5% CO₂ study alpha decreases at parietal sites were associated with increases in SBP and with the CO₂-induced increases in negative affect, thus suggesting a unique relationship between SBP and alpha band activation during the 7.5% CO₂ challenge. As the decreases in alpha activation were correlated with both the increases in SBP and self-reported negative affect, this suggests that 7.5% CO₂ inhalation has an equally pronounced effect on the autonomic arousal changes as well as cognitive or subjective aspects of anxiety. It is however intriguing that the challenge evoked an

opposing pattern of parietal alpha activation to the one described by Heller et al (1997), even though both studies observed increased autonomic arousal.

Less information is available in the anxiety literature regarding changes in alpha activation in regions other than frontal. A recent study by Tharawadeepimuk et al, (2014) measured alpha activity in experienced and amateur athletes prior to an important competition and reported that anxiety levels were associated with the quantity of alpha frequency band in posterior head region (parietal and occipital lobes) and that prior to an important competition, which can be seen as an anxiety-inducing stressor, less posterior alpha was observed than in the normal condition. In addition, amateur athletes have been reported to have a lower quantity of alpha frequency band than experienced athletes when the time of competition approaches. These results implicate the role of posterior regions in alpha activation in anxiety and stress, which is a finding that corresponds to the findings of the 7.5% CO₂ study presented here, which found significant decreases in alpha in parietal and occipital regions as compared to the normal air inhalation.

5.4.4 Gamma

No main effect of laterality was observed in the gamma band but an overall increase in activation across all left and right regions was observed during the 7.5% CO₂ challenge as compared to air. A main effect of gas, characterised by overall higher gamma band activation following the CO₂ as compared to air inhalation, and a significant gas x frontality interaction emerged. The CO₂ challenge resulted in significantly higher gamma activity across all sites, with this effect being greatest over occipital regions. In contrast the gamma elevation was least prominent in frontal regions.

The available gamma laterality literature in anxiety reports slightly different findings. Relatively more gamma power in the right temporal area is associated with positively valenced stimulus presentations and relatively more gamma in the left temporal area is associated with negative stimulus presentations (Müller et al., 1999). Oaths et al (2008) examined EEG gamma spectral power distributions during worry induction task and found that during worry induction, GAD patients showed higher levels of gamma activity than control participants in posterior electrode sites (parietal and occipital), previously associated with negative emotion. Specifically, there was more left posterior activity for worry and more overall gamma for the GAD compared to control participants. Moreover gamma fluctuations in these electrode sites were correlated with subjective anxiety changes, whereas in Study Four no changes in subjective anxiety (nor changes in autonomic arousal) were correlated with the increases in gamma, thus suggesting a potential limitation of 7.5% CO₂ inhalation as a model of GAD.

The role of parietal sites in gamma activation in anxiety has also been suggested by Miskovic et al (2009). In a sample of adults pre-selected for high and low social anxiety asked to prepare a speech, the authors found a significant main effect of condition (baseline and speech anticipation) for the parietal but not frontal sites, which was driven by an increase in gamma activity at the parietal electrode sites, from baseline to speech anticipation in both groups. This is interesting, as arguably, these results may suggest increases in gamma due to the speech preparation being a cognitive task, rather than a stressor, and there is evidence that suggests that gamma does increase during cognitive tasks, especially in the posterior sites. For example Fitzgibbon et al (2004) measured gamma activation in healthy subjects during eight cognitive tasks (Visual Checkerboard, Expectancy, Reading, Subtraction, Music, Expectancy, Word learning, Word recall, and a Video Segment) and found significant increases in gamma during all but the checkerboard task, in posterior but not frontal regions.

However, increases in gamma in both posterior and frontal regions have been observed due to the anxiogenic effects of 7.5% CO₂ challenge suggesting that frontal regions may be implicated in anxiety as opposed to cognitive task performance in terms of increased gamma activation and that studies should be careful in their design, as to whether the intended stressor may in fact be perceived as a mental task rather than anxiolytic stimuli. A study by Gemignani et al (2000) with healthy volunteers with simple phobia susceptible to hypnosis, revealed that during periods of hypnotic emotional activation (where participants were asked to produce a mental image of a phobic object) a significant increase in the gamma band with a left fronto-central prevalence was observed, coupled with significantly increased heart rate and respiratory frequency, which were not observed in periods of rest (during which the hypnotized subjects were asked to produce an emotionally neutral mental image). This also suggests a difference between activation during performance of a neutrally valenced mental task and negatively valenced mental task, which that can be perceived as a stressor, and implicates the role of frontal regions in gamma activation in anxiety.

In sum, it is interesting that the 7.5% CO₂ inhalation did not result in the more left posterior activity as compared to air, which was observed in a sample of GAD patients; and that the 7.5% CO₂ inhalation resulted in the additional increases in frontal regions. However regarding the finding that CO₂-induced overall increased gamma across all sites, it is not entirely surprising, as some literature suggests overall increases in gamma activation due to anxiety. For example when asked to imagine a phobic object, individuals suffering from a specific phobia show increases in gamma band activation as well as increases in heart rate and respiration (Gemignani et al., 2000). Also, gamma has been shown to decrease during periods of relaxation and to increase during periods of imagining negative emotional material (Sebastiani et al., 2003).

5.4.5 Theta

There were no differences in theta activation between left and right sites but 7.5% CO₂ inhalation resulted in overall significant increase in activity across both right and left sites as compared to air. No studies reporting asymmetry or any laterality effects in theta in anxiety have been identified thus the finding of no effect of laterality is not surprising. A significant main effect of gas, characterised by overall increases in theta band activation following the CO₂ as compared to air inhalation; a significant main effect of frontality, characterised by significantly lower theta (collapsed across inhalations) in the parietal relative to occipital and frontal regions; and a significant frontality x gas interaction were also identified. The 7.5% CO₂ challenge resulted in significantly higher theta activity across all sites, with this effect greatest at frontal regions.

These findings are unexpected. Current literature indicates that anxiety should be associated with decreases rather than increases in theta activation, for example Demerdzieva (2011) reported decreased theta in a sample of paediatric GAD relative to healthy controls, especially in central and midline regions. Moreover relaxation techniques such as meditation and mindfulness have been linked to significant increases in theta power (e.g. Lagopoulos et al, 2009). Lagopoulos et al (2009) found that significantly increased theta power was found for the meditation condition when averaged across all brain regions and theta was significantly greater in the frontal and temporal–central regions as compared to the posterior region. The pattern of results found in the 7.5% CO₂ study presented in this chapter mimics that commonly found in studies that aim to decrease rather than increase anxiety, despite the 7.5% CO₂ model being designed to induce anxiety. Intriguingly 7.5% CO₂ inhalation resulted in highest increases in theta being observed in the frontal regions, a finding similar to that of Lagopoulos et al (2009). This suggests that the theta activation pattern during the 7.5% CO₂ inhalation is similar to that experienced in healthy subjects during periods of meditation. One possible explanation comes from anecdotal evidence of subjective experiences during the CO₂ challenge, reported by some participants post-experiment. A number of participants spontaneously commented on the breathing noises associated with the challenge and feelings of concentrating on the breathing as well as feelings of drowsiness. These may be similar to experiences during meditation sessions.

More evidence that anxiety is associated with decreases rather than increases in theta comes from neurofeedback studies. As the literature broadly indicates that individuals with high anxiety display lower levels of both alpha and theta than healthy controls, a number of neurofeedback studies aimed to increase the levels of alpha and theta and subsequently reduce anxiety symptoms (e.g. Dadashi et al, 2015; see section 5.1). The results of Dadashi et al (2015) suggest that clinical anxiety is associated with low levels of alpha and theta in the occipital regions. In the

7.5% CO₂ study described in this chapter theta activation was significantly lower in the occipital regions than in frontal regions however, the anxiolytic inhalation has resulted in significant increases in theta in frontal, parietal and occipital regions as compared to air, rather than as expected, inducing decreases in those regions, suggesting an intriguing and unexpected effect of the 7.5% CO₂ model on the theta band activation.

5.4.6 The 7.5% CO₂ model

Evidence provides support for the 7.5% CO₂ challenge as a useful translational model which can clarify cognitive behavioural and biological mechanisms that underlie anxiety in humans (e.g. Bailey et al., 2007; Garner et al, 2011; Ainsworth and Garner, 2013). However no studies to date however attempted to observe brain activation changes during the 7.5% CO₂ challenge.

Here the 7.5% CO₂ inhalation induced anxiety, which was characterised by significant autonomic arousal as well as subjective and cognitive anxiety increases as compared to normal air inhalation, complemented by significant correlations between changes in subjective anxiety and changes in autonomic arousal due to the inhalation. CO₂ inhalation also induced changes to brain activation that are not otherwise observed in healthy individuals at rest during normal air inhalation.

As 7.5% CO₂ inhalation induces changes in autonomic arousal, it is important to establish that those changes are related to anxiety and that the changes in brain activation are not simply the result of increases of autonomic arousal. Aerobic exercise also commonly increases autonomic arousal and Wiese, Singh and Yuedall (1983) examined the effects of aerobic exercise on alpha power with subjects exercising on an ergometer for 40 minutes and alpha power measured before and after. They have found that alpha power has significantly increased following the exercise in occipital and parietal regions. As the 7.5% CO₂ challenge resulted in increases in autonomic arousal but decreases in parietal and occipital alpha, it can be seen that 7.5% CO₂ inhalation does more than just increase autonomic arousal.

Overall, these results suggest that 7.5% CO₂ inhalation induces alpha and gamma activation changes broadly consistent with those seen in anxiety but also induces other extra changes such as increases in frontal gamma thus implicating other regions not commonly implicated in anxiety. Moreover and unexpectedly, the model induces theta changes similar to those observed during relaxation and meditation.

There were however a number of significant associations between changes in subjective anxiety and changes in autonomic arousal. These were positive correlations between changes in SSAI, GAD7, PANAS negative affect and VAS negative affect and changes in SBP (r 's>0.46, p 's<.05), but

not HR or DBP, thus suggesting that SBP may be most affected by subjective anxiety during the 7.5% CO₂ challenge. However other CO₂ studies (e.g. Garner et al, 2011; 2012) reported different findings regarding the correlations between subjective anxiety and autonomic arousal induced by the CO₂-challenge. For example Garner et al (2011) found no association between the change in blood pressure and the subjective response to CO₂-challenge, but in contrast, reported a significant association between increased heart rate following CO₂ inhalation (relative to baseline) and increased state anxiety.

5.4.7 Comparison with other anxiogenic models (CCK-4)

Experimental panic induction with cholecystokinin-tetrapeptide (CCK-4³⁰) is an established model of panic disorder (e.g. Bradwejn & Koszycki, 2001), which stems from animal studies (Bradwejn & de Montigny, 1984). Subsequently CCK-related peptides have been shown to be anxiogenic in animal models of anxiety (e.g. Csonka et al., 1988; Palmour et al., 1992; Hendrie & Dovich, 1990). In humans, after intravenous administration CCK-4 dose dependently induces panic attacks in patients with panic disorder (e.g. Bradwejn et al., 1990) and to a lesser extent in healthy controls (range 0% to 70% (Rehferd, 1992; de Montigny, 1989; Koszycki et al, 1991; 1993; 1998; Shlik et al, 1997). The dose of 50 micrograms (µg) seems to give the most homogeneous response rate, ranging from 47% to 65% (Gilles et al, 2002). The CCK-4 induced panic symptomatology is accompanied by a marked somatic and neuroendocrine reaction which closely resembles spontaneously occurring panic attacks (e.g. Bradwejn et al., 1990; Bradwejn et al., 1991a).

CCK-4 is associated with increases in subjective states of fear and anxiety, increased autonomic arousal, as well as increased activation in the amygdala, insular cortex, claustrum, cerebellum, brain stem, and the ACC in healthy volunteers (Benkelfat et al, 1995; Eser et al, 2009; Javanmard et al, 1999; Schunck et al, 2006; Leicht et al, 2009). In addition, two studies reported dACC increases during anticipatory anxiety preceding the CCK administration (Eser *et al*, 2009; Javanmard *et al*, 1999). Apart from the bolus infusions, which produce panic attacks, continuous CCK-4 infusions (0.5 mg/60 min) have been established as inducing a stress response / mild acute anxiety. For example Shlik et al (1999) performed the acoustic startle response during a continuous intravenous administration of CCK-4 in healthy volunteers and found that in the first half of the infusion CCK-4 produced an increase of eye-blink startle amplitude from baseline values in contrast to the decrease observed at this time point with placebo. This was accompanied by a mild increase in anxiety and heart rate followed by fatigue as well as increases in plasma

³⁰ CCK-4 is synthetic analog of the endogenous neuropeptide cholecystokinin (CCK), which is suggested to act as a neurotransmitter (Rehfeld, 1985).

concentrations of adrenocorticotrophic hormone, cortisol, prolactin and growth hormone. Thus similarly to the anxiogenic effects of CO₂, CCK-4 can be used in different concentrations/dosages as either panic or acute anxiety/stress inducing.

To date only one EEG investigation have been reported in humans during the CCK-4 infusion. Knott et al, (2003) investigated, in healthy volunteers, the effects of continuous intravenous infusion of CCK-4 on EEG power asymmetry and power coherence³¹. Knott et al (2002) observed no significant treatment differences for absolute EEG power but, compared to placebo, CCK-4 infusion increased asymmetry and reduced coherence of slow-wave (delta) activity at midtemporal recording sites. Whereas the augmented delta power asymmetry may be interpreted as evidence of a hypoactivated left (vs. right) temporal lobe, the diminished delta coherence may be viewed as evidence of reduced left – right hemispheric communication.

Recently another study has investigated the roles of CCKA and CCKB receptors on CCK-4-induced anxietylike behaviors in mice using EEG (Li et al, 2013). Anxiety-like behaviors in mice were induced by an intracerebroventricular administration of CCK-4. The effects of CCKA and CCKB receptor antagonists (devazepide and CI-988, respectively) were examined using mouse anxiety tests (elevated-plus maze and light–dark box) and also by examining neuronal activities through EEG monitoring. They found that CCK-4 (3 g/kg of body weight) significantly induced mouse anxiety-like behaviors in the anxiety tests and also affected their EEG patterns, resulting in increase in spectral power and in relative power distribution in the delta and theta bands. These CCK-4 effects were completely suppressed by 1.0 mg/kg CCKB receptor antagonist, CI-988, while the same amount of CCKA receptor antagonist, devazepide was partly able to suppress the same effects, indicating that both CCKA and CCKB receptors are involved in in regulating anxiety-like behaviors in mice.

Both of the EEG CCK-4 studies suggest that in this model, the induced anxiety impacts on the delta frequency band, which has not been investigated in this chapter's 7.5% CO₂ EEG study. As both models are anxiogenic, perhaps it would be worth investigating the effects if the CO₂ challenge on the delta band, should this study be replicated. Interestingly, Li et al (2013) also implicated theta in the CCK-4 model and their finding (albeit in mice) suggests an increase in theta due to anxiety - a finding similar to that reported here for the 7.5% CO₂ challenge. As surprising a finding as the increase in theta was when looking at the 7.5% CO₂ model in isolation or when comparing to naturally-occurring anxiety, the similarity between the two anxiety models suggests that perhaps

³¹ Coherence data can indicate which particular brain areas are related / working together, with high coherence between two EEG signals being interpreted as evidence of a strong functional connection between two regions (e.g. Busk & Gailbraith, 1975; Callaway & Harris, 1974; Newton et al., 1993).

to a certain degree experimental models of anxiety result in similar brain activation, but differ from naturally occurring anxiety.

5.4.8 Future work/improvements

As this was a novel study, it would be desirable to replicate the study for possible validation of the study findings. As the sample was relatively small and reduced further due to extreme outliers, if the study were to be replicated a larger sample size should be sought, especially regarding the trends for main effect of frontality in gamma ($p=.07$) and main effect of laterality in theta ($p=.08$)

Various protocols report differing approaches to the choice of electrode groupings, for example some studies report only using F4 and F3 as frontal right and left electrodes (e.g. Coan & Allen, 2004; Thibodeau Jorgensen & Kim, 2006; Horan et al, 2014), whereas other protocols include a wider range to cover a broader area e.g. FC5, F3, F7 for left frontal and FC6, F4 and F8 for right frontal sites (e.g. Bruder et al, 1997). Other studies report activity in single electrodes without grouping them (e.g. Oathes et al, 2008). The lack of uniform consensus on the choice of electrode sites makes generalizing the results troublesome. Study Four grouped the electrodes consistently with Allen, Coan and Nazarian, 2004, into: left frontal (FP1, F7, F3) and right frontal sites (FP2, F8, F4), but either avoiding the somewhat arbitrary groupings in the future and reporting on individual electrode sites, or establishing a uniform grouping guide, could be considered in order to more accurately pinpoint what is happening during 7.5% CO₂ inhalation.

The non-invasiveness and low cost of EEG, its wide availability and ability to measure spontaneous brain activity, as well as the fact it requires a lower level of patient cooperation than fMRI attract researchers and clinicians to utilise this investigative tool (e.g. Badrakalimuthu, Swamiraju & de Waal, 2011). Despite these advantages, the EEG has a number of limitations. Spatial sampling in routine scalp EEG is incomplete, as significant amounts of cortex, particularly in basal areas, are not covered by standard electrode placement, and measuring changes in electrical activity in deep brain structures via EEG is difficult (e.g. Smith, 2005). Moreover the EEG procedure can be cumbersome and lead to difficult to interpret artefacts (e.g. Badrakalimuthu et al, 2011).

In contrast to EEG, which measure neuronal activity relatively directly by measuring changes in electrical activity as clusters of neurons become active (e.g. Crosson et al, 2010), fMRI relies on detecting changes in blood oxygenation and flow occurring in response to neural activity (i.e. a more active brain area consumes more oxygen, resulting in blood flow increases to the active area to meet this increased oxygen demand (e.g. Devlin, 2013). As EEG presents some limitations and fMRI offers an excellent spatial resolution (e.g. Devlin, 2013), it could be valuable to conduct a

complementary study, combining fMRI and EEG to comprehensively characterise the cortical and subcortical neural mechanisms of CO₂-induced anxiety.

A simultaneous EEG-fMRI acquisition can pose challenges as the signal quality in both modalities may decrease (Huster et al, 2012). The necessary introduction of conducting materials for EEG recordings in the environment of the magnetic resonance system may interfere with image acquisition (Mullinger et al., 2008), and similarly the induction of electromagnetic currents may degrade EEG signal quality (Kruggel et al., 2000; Debener et al., 2008; Yan et al., 2009). However, most of the technical difficulties have now been solved and commercial systems are now readily available (Kruggel et al., 2001; Debener et al., 2007; Mullinger et al., 2011). The introduction of the CO₂ kit into the already challenging EEG-fMRI set-up would certainly add to the complexity and difficulty of the experiment, however the ability to noninvasively record human brain activity with both high spatial and temporal resolution that the simultaneous EEG-fMRI experiment would offer would be highly valuable.

vi. Chapter 6

General Discussion

The programme of work described within this thesis had two main aims: first, to explore the correlates of two novel approaches for treating anxiety (tDCS and ABMT), and second, to provide further investigation of the effects of 7.5% CO₂ inhalation by examining its effects on brain EEG.

6.1 tDCS

The results of Study One and Study Two (Chapter 2 and 3) contribute to the growing body of literature regarding the effects of acute administration of tDCS on cognition, mood and autonomic arousal. These studies extend previous findings and for the first time provide an investigation of the effects of anodal tDCS of left DLPFC on subjective anxiety as well as on neurocognitive aspects of anxiety, such as hypervigilance to threat within an experimental model of anxiety.

6.1.1 Mood and subjective anxiety

In Study One (ANT) no acute effects of anodal tDCS of the left DLPFC on mood were found, (across multiple measures) suggesting that the observed enhancement of executive control following the active anodal tDCS occurred independently of mood. In both groups post-tDCS decreases in negative affect but also decreases in positive affect were observed. Study Two yielded similar results, with no significant mood changes but with overall decreases in positive affect post-tDCS in both the active and sham groups. Collectively these results suggest that a single 20 minute session of anodal tDCS of IPFC does not alter current mood in healthy volunteers.

Study Two extended previous findings and for the first time examined the effects of anodal tDCS of left DLPFC on subjective anxiety. No significant changes in subjective anxiety were observed - a single dose of anodal tDCS did not significantly alter levels of anxiety post-administration, nor in response to 7.5% CO₂ challenge. Similarly no effects of tDCS on subjective anxiety were found in unchallenged individuals (Study One), with minor decreases in anxiety reported post-tDCS across groups. These results suggest that a single session of active anodal tDCS does not alter subjective anxiety levels, or the anxiogenic response to CO₂-challenge. These null results converge with evidence from other acute administration protocols, which report null effects of acute

administration of tDCS to the DLPFC on mood in healthy volunteers (e.g. Fregni et al, 2008; Plazier et al, 2012; Brunoni et al, 2013). Studies that do report positive changes in mood using tDCS in healthy volunteers as well as with depressed patients have reported those effects following a multiple rather than single doses of tDCS administration (e.g. Palm et al, 2012) (see Chapter 2, section 2.4.3, and Chapter 3, section 3.4.1). Limited research concerning the effects of tDCS on mood examining sites other than PFC (e.g. the occipital area³²) suggests that bioccipital tDCS similarly does not alter mood in healthy volunteers in an acute administration (Plazier et al, 2012). Collectively these studies combine with the results of Study One and Study Two and suggest that in order to elicit discernable improvements in mood repeated administration tDCS protocols may be required.

6.1.2 Autonomic arousal

Study One revealed no acute effects of the active anodal tDCS of the left DLPFC on autonomic arousal (HR, SBP, DBP) suggesting that the observed enhancement of executive control following the active anodal tDCS occurred independently of changes in autonomic arousal. Post-tDCS both groups experienced a significant decrease in HR as compared to baseline, which could perhaps be attributed to the acclimatisation to the lab setting. Study Two found that 20 minutes of active anodal tDCS did not modify HR or SBP, but did achieve a small reduction in DBP, an effect that warrants replication.

Results from Study One and from HR and SBP analyses in Study Two correspond with previous tDCS studies which also report null effects of acute tDCS administration (of other cortical sites) on blood pressure and heart rate (e.g. De Vries et al, 2010; Raimundo, Uribe & Brasil-Neto, 2012; Vandermeeren et al, 2010) (see Chapter 2, section 2.4.3, and Chapter 3, section 3.4.2). Despite reporting no effects of active tDCS on blood pressure or heart rate, both active and sham groups were reported to display significant changes on other measures of arousal, such as increases in galvanic skin response and cortisol levels (e.g. Raimundo et al, 2012), suggesting a stress response to the experimental sessions. In contrast across groups in Study One and Two all autonomic arousal measures post-administration somewhat decreased, suggesting an overall calming rather than stress-inducing effect of the 20 minutes session.

³² The occipital region was chosen as the target area based on results by Thimineur et al (2007) who demonstrated that stimulation of the greater occipital nerve (C2) with an implanted stimulator improved pain scores and mood in patients with fibromyalgia. However, the mood improvements may have been correlated with the decreases in pain, rather than be caused by the stimulation per se.

6.1.3 Cognition

Study One demonstrated that executive attention was greater following 20 minutes of active anodal tDCS of the left DLPFC than sham tDCS. Both groups performed faster on the “easy” congruent trials as compared to the incongruent trials however the difference between the incongruent and congruent trials was significantly smaller in the active group (i.e. tDCS was followed by reduced distractor interference in the flanker task). These findings support evidence implicating the role of the prefrontal cortex in attention control (e.g. Bishop et al, 2004). No effects of tDCS on alerting or orienting networks were observed.

These results complement findings from a number of recent studies in healthy volunteers, suggesting positive effects of tDCS on cognition, including inhibitory control, emotional face recognition, selective attention and attentional bias acquisition (e.g. Hsu et al, 2011; Nitsche et al, 2012; Gladwin et al, 2012; Clarke et al, 2014) (see Chapter 2, section 2.4.1). Study One provides no evidence that left frontal tDCS alters alerting and orienting attention functions and these findings differ from previous findings using the ANT (e.g. Coffman et al. 2012) which found improved alerting, but not orienting or executive control due to anodal tDCS. However, these differences can be attributed to different target areas (right inferior frontal cortex vs left DLPFC) especially as the alerting network has been linked to the activation of frontal and parietal regions (Coull, Nobre & Frith, 2001; Fan et al, 2005) and executive control to activation of the ACC and lateral prefrontal cortex (Bush, Luu & Posner, 2000; Fan et al, 2005).

Despite growing evidence of positive effects of tDCS on cognition, recent controversy and debate over the positive effects of tDCS on cognition arose, especially regarding the number of tDCS sessions needed to observe the effects. Horvath et al (2015) conducted a large quantitative review of the cognitive data available to date, which included 59 studies with the poolable outcome measures divided into four categories: executive function, language, memory and miscellaneous. None of the 59 analyses provided evidence that tDCS can modulate cognition in an acute administration. However, recently Price et al (2015) performed an in-depth review of Horvath et al (2015) meta-analysis and identified substantial methodological issues, including inconsistent and inappropriate data selection, mischaracterisation of examined studies, incorrect subject numbers, and problematic statistical analyses, which systematically produced a misleading picture of tDCS findings. Combined with the lack of a power analysis the results questioning the efficacy of tDCS in cognition in an acute administration should be taken with caution.

Study Two extended study One to examine whether tDCS can modulate cognition during the CO₂ challenge. Specifically it examined whether 20 minutes of left anodal tDCS of DLPFC can improve

attention control and reduce erroneous eye-movements to threat in an antisaccade task during CO₂-induced anxiety. Results indicated that in healthy individuals the active tDCS resulted in significantly lower error rates on threat as compared to neutral trials (across pro- and antisaccade trials) with no such difference observed in the sham group. The active tDCS group was predicted to display a superior (more accurate) performance on the antisaccade as compared to pro-saccade trials, with this effect greater for threat relative to neutral images. This trend has been observed but did not reach statistical significance, which warrants replication.

The results of Study Two also indicate that both groups performed faster on pro- as compared to anti-saccade trials and that tDCS did not modify the latency to make correct eye-movements. However there was a strong trend for the active tDCS group to be slower to attend to the threat in the pro-saccade trials relative to the sham group (slower orienting to threat). A faster disengagement from threat was predicted (faster correct antisaccade latencies). Even though this was not observed, this study provides evidence that active anodal tDCS can change patterns of attention to threat and away from threat.

As no other studies have investigated the effect of tDCS on antisaccade task performance, direct comparisons are not possible; however the findings from Study Two complement evidence from studies using other brain stimulation modalities (TMS) and other emotional attentional tasks that index selective attention to threat (e.g. De Raedt et al, 2010) (see Chapter 3, section 3.4.3), suggesting that stimulation of the left DLPFC results in diminished attention to threat. Moreover the latency finding in Study Two, which suggests that active tDCS of DLPFC can result in slower initiation of pro-saccades, accords with previous TMS DLPFC findings (e.g. Nagel et al, 2008, who found increase in saccade latencies post TMS). Additionally, the results of Study Two are in line with the recent findings of a tDCS dot probe study, in which Ironside et al (2015) demonstrated that 20 minutes of active anodal tDCS of left DLPFC (bipolar-balanced montage) can result in reduced vigilance to threatening stimuli (relative to sham and bipolar-unbalanced montage, where the cathode is placed on the supraorbital ridge), with no other effects on emotional processing. Collectively these results suggest that stimulation of left DLPFC by TMS and/or modulation by anodal tDCS can result in more accurate disengagement from and in slower engagement to threatening stimuli.

Consequently evidence from Study One and Study Two suggests that this tDCS protocol affects neither mood nor subjective anxiety in unchallenged individuals, but also not in those experiencing elevated levels of experimentally induced anxiety. This suggests that the effect of 20 minutes of left anodal tDCS of DLPFC predominantly affects cognitive rather than subjective or

autonomic characteristics of anxiety, and may potentially be effective in improving maladaptive biases in attention control and threat processing in clinical anxiety.

6.1.4 Limitations and future directions

Both studies suffer from some limitations, the main one being the lack of an off-target active control condition (OAS). OAS works by delivering a full dose of stimulation to “an area of the scalp where it is assumed to be unlikely to affect the process being studied” (Davis et al, 2013, p. 2974). The use of an OAS could be considered alongside the use of the sham condition. Due to blinding in sham tDCS trials, especially with relatively low current densities, not always being successful (e.g. O’Connel et al, 2012) it may be more effective to compare the active condition with an OAS, where unlike in a typical sham condition, the sensation of the current being delivered will be felt throughout the session rather than in the first 15 seconds only. As the blinding procedure was not successful in Study One (80% of participants in both groups correctly identified the tDCS type they had received) the use of an OAS would have been particularly useful.

Due to the positive results obtained in Study One and Two regarding executive control and engagement and disengagement from threat, the use of tDCS could now be considered for use with high trait anxious or clinically anxious individuals to potentially target the attentional bias to threat that is common in patients with GAD. Perhaps a repeated rather than acute administration protocol should be employed, a suggestion informed by the lack of improvements in subjective anxiety or mood following an acute administration in Study One and Two, and the evidence of positive effects of repeat anodal tDCS protocols in depression (e.g. Fregni et al, 2006; Rigonatti et al, 2008), whereby improvements in the range of 28.4% - 60% in symptoms and self-report depression scores were reported with 20 minutes of both 1 mA (e.g. Fregni et al, 2006) and 2mA (e.g. Loo et al, 2012) anodal tDCS of the left DLPFC, in at least 5 sessions.

Moreover the use of other tasks and outcome measures (e.g. startle eyeblink reflex) could be considered in future tDCS studies. The startle reflex is an automatic protective response (a rapid muscular contraction) elicited by an abrupt and intense stimulus (e.g noise) (Poli & Angrilli, 2015). It can be measured by the extent of a noise-triggered eyeblink, and considered a valid non-invasive tool for studying attention and emotion (e.g. Angrilli et al., 1996, 2008; Orr et al., 1995; Grillon et al., 1996; Ludewig et al., 2005). Recently Poli and Angrilli (2015) demonstrated that greater general startle magnitude is related to higher state anxiety levels. This suggests that the startle eyeblink reflex could be used as a valid tool for studying the neural excitability underlying anxiety and emotional dysfunction. A tDCS study utilising the current protocol could measure

participants' baseline startle magnitude and investigate whether 20 minutes of 2 mA anodal tDCS results in decreases in startle magnitude post-administration (relative to sham).

Lastly, protocols examining tDCS effects in other cortical sites could be considered in the future. Recently investigations of ctDCS (cerebellar transcranial direct current stimulation) have gained popularity (e.g. Grimaldi et al, 2014), particularly in the context of neurological conditions. However the cerebellum has also been identified as part of the network that processes complex emotional facial expressions (Fusar-Poli et al, 2009). A recent study reported that both anodal and cathodal ctDCS significantly enhanced the response to negative facial emotions (with no effect on positive or neutral facial expressions), suggesting that ctDCS can affect responses in cognitive and emotional tasks, and could be considered in future use.

6.2 ABMT

Study Three found that ABMT training did not modify attentional bias. This null effect adds to the growing evidence that questions the robustness of ABM training by visual probe paradigms (e.g. Carlbring et al, 2012; Boettcher et al, 2012; Boettcher et al, 2013; Neubauer et al, 2013) (see Chapter 4, section 4.4.1). Without a training-induced change in attention, a change in emotion cannot be expected and thus collectively these studies represent 'manipulation failures', rather than evidence against any potential therapeutic value of ABMT. A recent review by Clarke et al (2014) suggest that studies which fail to modify both selective attention and emotional vulnerability provide reassurance of the sound theoretical basis for ABM. Thus collectively these studies support the theory that successful bias modification leads to consequent change in emotional vulnerability but failure in modification leads to no change. More research into any potential use of ABMT in anxious individuals should be conducted.

6.2.1 Limitations and future directions

ABMT studies use various methodologies and report varying results. One or a combination of methodological differences between Study Three and other published studies could perhaps explain the failure of Study Three to train attention.

It has been suggested that studies which use verbal target stimuli (words) presented in a top-bottom formation generate significantly larger effects than face stimuli or stimuli presented in a side-by-side formation (Hakamata et al, 2010). Moreover greater numbers of training sessions (e.g. 8 sessions Amir et al 2009a, 2009b; 4 sessions Bar-Haim et al, 2011) result in increased effects of ABMT (Hakamata et al, 2010). Lastly ABMT protocols were shown to yield significantly larger effect sizes in clinical than healthy populations (Hakamata et al, 2010).

In sum this suggests that ABMT consisting of word stimuli presented top-to-bottom is most effective in clinical and highly anxious populations, in repeated session protocols. Study Three used the more effective ABMT type and display, but in a single session protocol, in a healthy sample with no existing baseline attentional bias. This suggests that the failure to modify bias in Study Three may largely be due to the sample characteristics (floor effects).

As a measure of selective attention, the visual probe task has recently been criticised (e.g. Cooper et al, 2011; Kappenman et al, 2014) due to its problems with reliability. Consequently alternative tasks could be considered for use in attention training. Recently Notebaert, et al (2014) developed a new person-identity-matching (PIM) task (see section 4.4.3), which encourages selective attention toward or away from threat, and the study reported success in both modifying attentional bias, and impacting on emotional reactivity.

In Study Three the training was not administered during a state of increased anxiety – i.e. ABMT was performed prior to 7.5% CO₂ challenge. In the future, use of CO₂ inhalation to induce anxiety simultaneously to the ABMT training could be considered, paired with the use of a different stress-inducing task post training (e.g. a speech or an anagram task used in other studies) in order to examine the effects of ABMT on anxiety reduction. Attempting to modify attention in a CO₂-induced anxious sample could potentially provide results more similar to those seen in clinical samples.

Future studies could furthermore explore a combination of ABMT and tDCS to yield optimal results, informed by the recent findings reported by Clarke et al (2014) whereby left anodal DLPFC tDCS during either an “attend threat” or “avoid threat” ABMT in healthy volunteers led to greater evidence of attentional bias acquisition in the targeted direction (toward or away from threat) in the active as compared with sham group. These results provide evidence that increasing activity in the DLPFC leads to greater evidence of attention bias modification, and suggest that a combination of the two treatments (tDCS and ABMT) could yield optimal effects.

6.3 Validation of the 7.5% CO₂ model

This thesis contributes to the body of literature validating the 7.5% CO₂ model, by a) providing further evidence of a robust effect of the CO₂ inhalation on subjective anxiety and autonomic arousal in Study Two, Three, and Four, and b) by evaluating novel interventions for generalized anxiety applied immediately prior to the CO₂ challenge (neither tDCS nor ABMT had previously been used with the CO₂ model). Moreover the thesis examined for the first time the effects of CO₂ challenge on EEG brain activity at rest.

The 7.5% CO₂ model of generalized anxiety is described in detail in Chapter 1 (section 1.7.1). To date, evidence suggests that inhalation of air enriched in levels of CO₂ to 7.5% can increase subjective anxiety and autonomic arousal (Bailey et al. 2005; Bailey & Nutt 2008; Bailey et al. 2011a; Poma et al. 2005), and induce neuropsychological biases in attention and emotion processing that characterise clinical anxiety (e.g. Ainsworth & Garner, 2013; Garner et al, 2011), demonstrating that CO₂ can induce hypervigilance towards and deficient inhibition of visual threat stimuli. Moreover recently studies found that both established pharmacological treatments for anxiety (e.g. (SSRI) paroxetine) and psychological interventions (e.g. mindfulness techniques) can reduce CO₂-induced anxiety in healthy humans (e.g. Bailey et al., 2007; Ainsworth et al, 2015) (see Chapter 3, section 3.1). Study Three has found that tDCS can modulate cognitive but not subjective aspects of CO₂-induced anxiety in healthy humans. Taken together these findings suggest that the 7.5% CO₂ challenge is considered a valid human experimental model of subjective, autonomic and neuropsychological features of anxiety, with capacity to evaluate therapeutic interventions, prior to phase-II/III clinical trials in patient populations (Bailey et al., 2011a).

Specificity of the model to generalised anxiety

The 35% CO₂ inhalation induces acute symptoms of anxiety, more panic-like and akin to extreme state anxiety, whereas the 7.5% CO₂ inhalation can be seen as a more trait-like anxiety model (see section 1.7). The symptoms that the 7.5% induces in healthy volunteers mimics chronic, more generalised anxiety. The main symptoms of the 7.5% CO₂ challenge include respiratory symptoms (e.g. breathing heavily, hyperventilation) and cardiovascular symptoms (e.g. increases in heart rate and blood pressure). These symptoms alone may also be symptoms of acute stress (APA, 2015) and in fact recently some researchers begun to refer to the 7.5% CO₂ model as a model of “acute stress and anxiety” (e.g. Brühl et al, 2015; van Ghesel Grothe et al, 2015).

The increases in physiological arousal alone, or subjective anxiety and worry feelings alone are not in itself exclusive to GAD and could be observed in other disorders. It is however the combination of experiencing both subjective, autonomic and cognitive anxiety symptoms in an absence of a threat stimulus, continuously for the whole duration of the inhalation; to a level lower than that observed in a PD that lends support to the 7.5% CO₂ model as GAD specific. For example in phobic patients the specific phobia is the source of anxiety and worry and the phobic stimulus triggers the anxiety – in GAD the anxiety and worry are non-target specific and experienced continuously throughout the day. Similarly the 7.5% CO₂ inhalation induces feelings and symptoms without presenting any threat stimuli. Furthermore, despite anxiety and depression sharing some neurocognitive processes, the 7.5% CO₂ model can induce hypervigilance to threat (e.g. Garner et

al, 2011) which is a hallmark of anxiety but not depression (e.g. Williams et al, 1988) in healthy volunteers.

Moreover anxiolytic medication commonly prescribed for generalised anxiety (e.g. lorazepam) has been shown to significantly reduce peak CO₂-induced subjective fear, feelings of wanting to leave, tension and worry in healthy volunteers undergoing the 7.5% CO₂ challenge (single dose; 2mg) compared to placebo (Bailey et al, 2007). This suggests that this CO₂ model of anxiety is sensitive to lorazepam and gives support to its utility as an experimental model of general anxiety disorder in healthy volunteers.

With this in mind, the results of Study Two, which suggest that left anodal tDCS may help target the maladaptive attentional biases in anxiety, could be viewed as encouraging for the use in GAD populations. However even though the 7.5% CO₂ model is GAD specific, the attentional bias to threat is not exclusive to GAD and has been demonstrated in other populations as well, for example: in individuals with high trait anxiety (e.g. Broadbent & Broadbent, 1988; Mogg et al., 1994), patients with OCD (e.g. Cohen et al., 2003; Lavy et al., 1994), PTSD (e.g. Foa et al., 1991; Kaspi et al., 1995), social phobia (e.g. Mattia et al., 1993) and specific phobias (e.g. Lavy et al., 1993). An attentional bias for relevant items has moreover also been demonstrated in substance users (e.g. Field et al., 2006), chronic pain patients (e.g. Roelofs et al., 2002), smokers (e.g. Field et al., 2009), and eating disorder patients (e.g. Dobson & Dozois, 2004). This suggests that tDCS may be used to target the attentional bias in other populations as well.

6.3.1 EEG

No published studies to date have examined brain EEG during 7.5% CO₂ challenge.

Overall, Study Four suggests that 7.5% CO₂ inhalation induces alpha and gamma band activation changes broadly consistent with those seen in anxiety (e.g. Heller et al, 1997; Mathersul et al, 2008; Oathes et al, 2008). Autonomic changes due to anxiety have been linked with greater right frontal alpha activation, and subjective and cognitive anxiety with a greater left frontal alpha activation - Study Four found larger alpha activation in both left and right frontal regions as compared to air, corresponding to the observed CO₂-induced increases in autonomic arousal and subjective anxiety. Moreover significant differences were found between air and CO₂ inhalation in parietal and occipital regions, with CO₂ resulting in significantly lower activation of those regions as compared to air, with these activation changes correlated with the changes in autonomic arousal (systolic blood pressure) and mood (negative affect).

The 7.5% CO₂ inhalation led to significant increases in gamma power in parietal, occipital and frontal regions as compared to air, with this effect highest in occipital regions. The results were similar to previous evidence of elevated posterior gamma activity in GAD patients (e.g. Oathes et al, 2008) and of left fronto-central increase in gamma in phobic volunteers (e.g. Sebastiani et al, 2000). Moreover other studies suggest overall increases in gamma activation due to anxiety (e.g. Gemignani et al., 2000; Sebastiani et al., 2003) in phobic individuals when asked to imagine a phobic object.

Study Four found an unexpected pattern of theta activation during the CO₂ challenge, namely overall significant increases in theta activation across all sites. The 7.5% CO₂ challenge was thus found to mimic results commonly found in protocols aimed to decrease rather than increase anxiety (such as during meditation, e.g. Lagopoulos et al, 2009).

6.3.2 Limitations and future directions

The choice of electrode groupings varies across study protocols, with some studies reporting only F4 and F3 as frontal right and left electrodes (e.g. Coan & Allen, 2004; Thibodeau Jorgensen & Kim, 2006; Horan et al, 2014), others including a wider range covering a broader area e.g. FC5, F3, F7 for left frontal and FC6, F4 and F8 for right frontal sites (e.g. Bruder et al, 1997) and others still avoid groupings altogether and report activity in single electrodes (e.g. Oathes et al, 2008). Consequently, this lack of a uniform choice of electrode sites makes generalisation difficult. Study Four used electrode groupings consistent with Allen et al (2004) with FP1, F7, F3 for left and FP2, F8, F4 for right frontal sites, however in the future studies should aim to employ a uniform grouping system in order to make this process less arbitrary and to improve generalizability.

Despite many advantages (e.g. the non-invasiveness, low cost, wide availability and ability to measure spontaneous brain activity) the EEG has a number of limitations, such as incomplete spatial sampling in routine scalp EEG (e.g. Smith, 2005). As fMRI offers an excellent spatial resolution (e.g. Devlin, 2013), it could be of value to conduct a complementary study, combining fMRI and EEG to comprehensively characterise the cortical and subcortical neural mechanisms of CO₂-induced anxiety. As the neurocognitive models of anxiety discussed in section 1.1.6 (e.g. Bishop 2004; 2006; 2007) suggest that anxiety may be characterised by an interplay of reduced activity in DLPFC and a hyperactivity of the amygdala, the 7.5% CO₂ challenge could be expected to induce changes consistent with that view. These could be demonstrated via fMRI, which would supplement the spectral power EEG results.

As highlighted in section 5.4.8. a simultaneous EEG-fMRI recording during the CO₂ challenge would potentially pose technical and functional difficulties. The presence of artefacts such as

head movements and large changes in blood flow produced by CO₂ can be challenging (Colasanti, 2012). However these limitations can be overcome and in 2012 an fMRI investigation of brain responses to CO₂ challenges in subjects with high and low CO₂-vulnerability was underway at the University of Maastricht (Colasanti, 2012).

Overall, this novel study yielded interesting and unprecedented results and it would be desirable to replicate these for further validation. Moreover future studies could consider conducting direct comparisons between healthy individuals undergoing 7.5% CO₂ challenge and a sample of GAD patients in order to examine whether the brain activity at rest is comparable between experimentally-induced and clinical anxiety.

Furthermore, guided by the EEG findings of other experimental anxiety models such as the CCK-4 (e.g. Knott et al, 2002; Li et al, 2013) it seems reasonable to suggest that in the future the role of delta band during the 7.5% CO₂-induced anxiety should also be investigated.

6.4 Summary and implications

Collectively the results of Studies 1-4 extend our understanding of the effects of left anodal DLPFC tDCS on cognition, mood, subjective anxiety and autonomic arousal. Furthermore this thesis provides initial evidence that 7.5% CO₂ inhalation might model patterns of EEG activity commonly observed in trait anxious/clinical samples. The results suggest that tDCS may be a useful tool in targeting the cognitive aspects of anxiety but that perhaps repeated administration protocols should be used in order to elicit mood and subjective anxiety changes.

The thesis also revealed that the use of ABMT in healthy samples presenting no baseline attentional bias may be affected by floor effects so that this intervention may be most suited for high trait anxious/clinical populations. Moreover more research should be conducted to identify best ABMT training tasks, which may be better placed and/or more reliable to measure selective attention than the recently criticised visual dot probe task (e.g. Cooper et al, 2011; Kappenman et al, 2014). The findings included within this thesis are relevant to research that aims to improve existing interventions for generalised anxiety and aims to contribute to the debate regarding optimal use of those two novel treatment candidates in anxiety.

Appendices

A.1 tDCS KIT

The tDCS stimulators were powered by 2 AA batteries per stimulator. 2 skin surface electrodes (red – left – anodal; black – right – cathodal), spongy pads to enclose the electrodes, saline solution (to soak the pads and prevent skin irritation, zero-gel and a self-cohesive bandage (to keep the electrodes in place) were used (see *Figure 13* for the image of the kit).

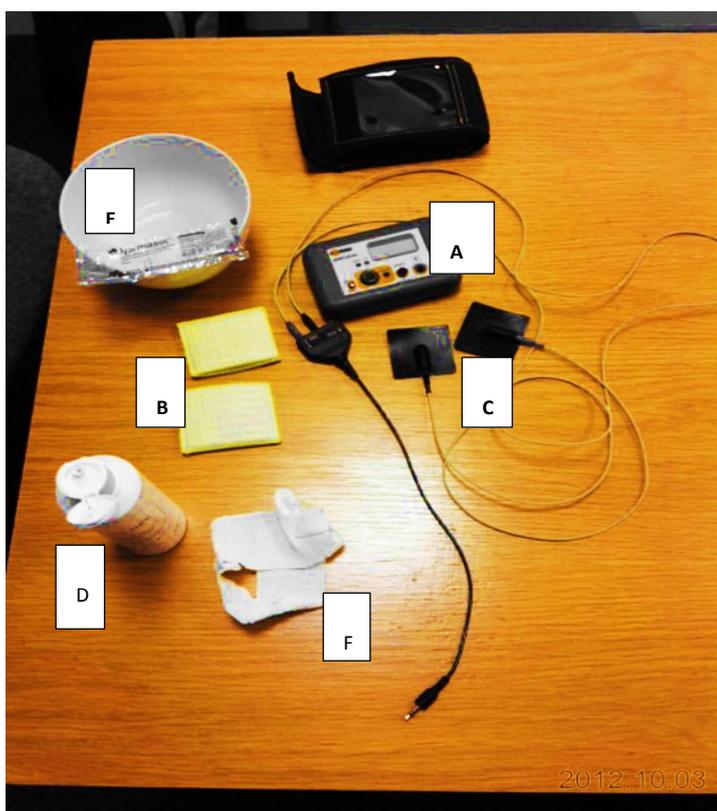


Figure 13 The tDCS kit.

A = stimulator; B = sponge pads; C = electrodes; D = zero-gel; E = saline solution; F = self-cohesive bandage

The preparation of the equipment was done in full view of the participant (see *Figure 14* for images of the preparation). 2 special sponges with pockets for encasing the electrodes were soaked in normal saline solution (to prevent any skin irritation). The zero-gel was applied to both the electrodes on the side that would be touching the participant's skin. The electrodes covered in gel were then placed in the soaked spongy pockets and a further drop of gel was placed on the spongy pads. With the electrodes inside the pads, the procedure was as follows: the red anodal electrode was placed on the left side of the participant's forehead, aiming to stimulate the dorsolateral prefrontal cortex; and the black

cathodal electrode was placed on the right side of the forehead aiming to stimulate the right dorsolateral prefrontal cortex. The participant was asked to hold on to the electrodes with their hands for a brief moment and the researcher prepared a strip of a self-cohesive bandage and placed it over the electrodes, across the forehead and around the total circumference of the head, fastening it together in order for the electrodes to firmly stay in place for the duration of the tDCS administration. The electrodes' cord was then plugged in to the stimulator and the device was then turned on. The researcher would ask if the participant was ready and then press the start button. The device would then display a timer going down from 20 minutes to 0. The researcher would stay present in the room throughout the entire administration period.

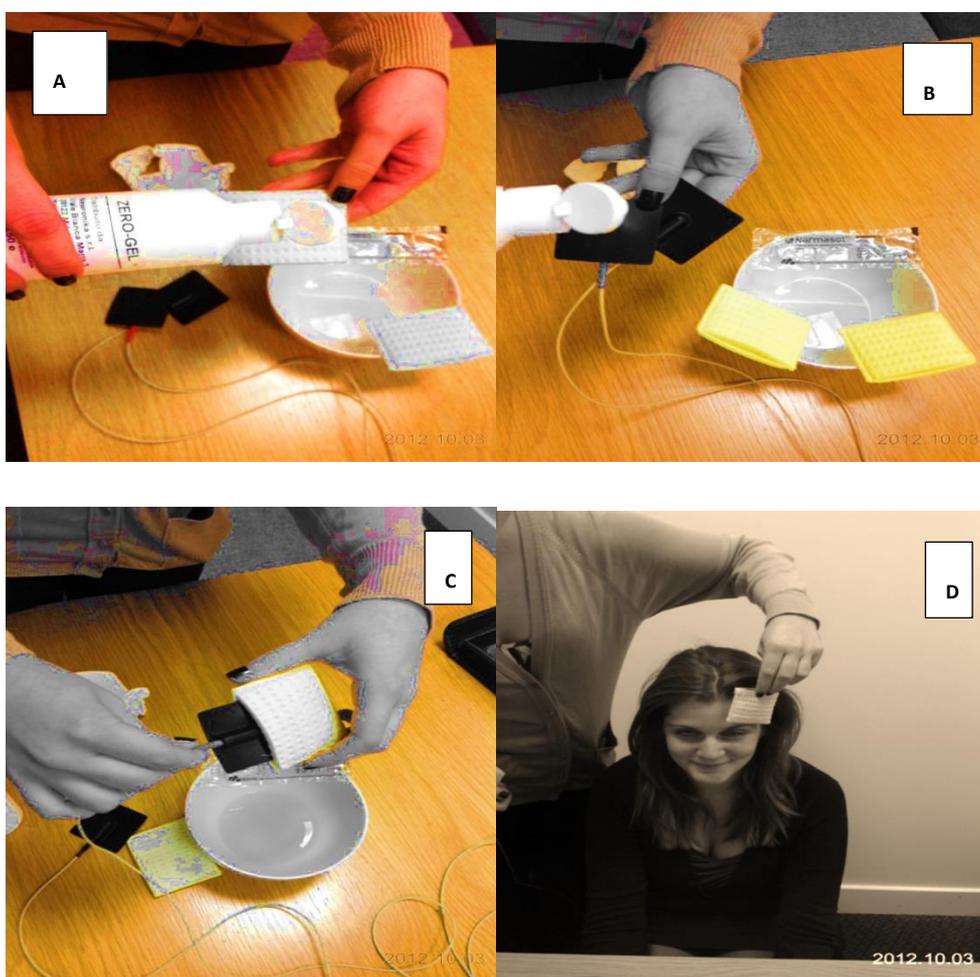




Figure 14 Preparation of the participant for the tDCS administration

A = applying zero-gel on the soaked pads; B = applying zero-gel on the electrodes; C = placing electrodes inside the pads; D = placing electrodes onto participant's forehead; E = securing the electrodes with a self-cohesive bandage; F=participant ready for the tDCS administration.

A.2 TELEPHONE SCREENING CRF (v1 19/09/12)

Subject Identifier		Subject Initials		Day	SCREENING				
Date of Visit (dd/mm/yyyy)			/		/				

[Note identical to form in study ID = - RGO approval
26/10/10]

Telephone Interview

STUDY :

**EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON
ATTENTIONAL BIASES AND COGNITIVE PERFORMANCE**

DEMOGRAPHICS

<u>Name</u>														
<u>Gender (✓)</u>	<u>Male</u>			<u>Female</u>										
<u>Race (✓)</u>	<u>White</u>			<u>Black</u>			<u>Asian</u>			<u>Other (specify)</u>				
<u>DOB (dd/mm/yyyy)</u>			<u>/</u>			<u>/</u>						<u>Age (yrs)</u>		
<u>Address</u>														
<u>Tel. Home</u>														
<u>Tel. Work</u>														
<u>Mobile</u>														
<u>Email</u>														

CAFFEINE CONSUMPTION

Does the subject consume caffeine-containing drinks? (✓)	YES		NO		
<i>If YES,</i>	Coffee	Tea	Cola	Cocoa	Total
Number of cups/ day					

If consumes more than 8 caffeinated drinks per day, screening stops here

ALCOHOL CONSUMPTION

Does the subject drink alcohol? (✓)	YES		NO	
<i>If YES,</i>	Beer	Wine	Spirits	Total
Number of units/ week				

If consumes more than 50 units/week (male) or 35 units/week (female), screening stops here

MEDICAL HISTORY – self-report

Are you alcohol/ drug dependent?
Do you suffer from migraines?
Do you suffer from a high blood pressure?
Do you suffer from a skin condition (particularly on the face/head area)?
Do you suffer from epilepsy?
Do you have a G.P. at the moment?
Do you use contraception?
Do you have any electronic or metal implants?
Any relevant information you think is important?
Do you agree to have your contact details kept in our database, so we can contact you regarding future studies?

A.3 SET UP OF THE CO₂ CHALLENGE



Figure 15 Lab set up for the 7.5% CO₂ challenge.

A.4 SCREENING CRF (v1 19/09/12)

Subject Identifier		Subject Initials		Day	SCREENING
Date of Visit (dd/mm/yyyy)			/		/

[Note identical to form v2 in study ID = 150 - RGO approval 26/10/10]

Study Day

STUDY :

EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON ATTENTIONAL BIASES AND COGNITIVE PERFORMANCE

Principal Investigator:

Dr Matthew Garner, University of Southampton

INFORMED CONSENT

	Time (24hr format)	YES	NO
Information sheet read			
Consent sheets signed	:		

ALCOHOL BREATH TEST

Alcohol breath test	Time (24 h format)		:	
Result (✓)	Negative		Positive	

If POSITIVE, study stops here

Drunk caffeinated beverage in last 12 hours (✓)	Yes		No	
If yes	Time (24 h format)		:	

CAFFEINATED BEVERAGES

CONCOMITANT MEDICATION

Has the subject used any medication since the telephone screening? (✓)	YES		<i>If YES, Report and see below</i>
	NO		

Note here:

PRIOR MEDICATION

Medication NOT permitted: any medication within 8 weeks prior to study start.

Exceptions: Local treatment, oral or injectable contraceptives, stable (3 months +) HRT, occasional aspirin or paracetamol.

CONCOMITANT MEDICATION

Local treatment, and occasional paracetamol or aspirin are permitted

CARDIOVASCULAR ASSESSMENT

<i>Blood Pressure and Heart Rate</i>	Time (24hr format)				Systolic BP (mmHg)	Diastolic BP (mmHg)	Pulse Rate (beats/min)
Sitting			:				

ELECTRONIC / METAL IMPLANT ASSESSMENT

Has the subject got any electronic implants (e.g. pacemaker / implanted defibrillator)? (✓)	YES		<i>If YES, Report and see below</i>
	NO		
Has the subject got any metal implants? (✓)	YES		<i>If YES, Report and see below</i>
	NO		

PREGNANCY SCREEN

Because the effects of tDCS on an unborn child or breast milk are currently not known; you should not take part in this study if you are pregnant or breast-feeding.

Therefore, by signing this declaration you are confirming that one of the following 4 statements applies:

- I am using a reliable form of contraception (see below)*
- I have not had unprotected sex since my last menstrual period
- I have not had unprotected sex since taking emergency contraception
- I have been sterilised, or have reached the menopause

**i.e. one of the following: Barrier methods and spermicide; Oral contraception (the Pill); Intrauterine contraception (IUD – known as a coil); Injectable contraception; Implant; or Contraceptive patch*

I also confirm that I am not breastfeeding.

Participant Signature..... Date.....

Print name.....

Experimenter signature..... Date.....

Name.....

Pregnancy inclusion criteria met: Yes No (no further participation)

Height (cm)		

Weight (kg)			
		.	

BMI (kg/m ²)				
		.		

NEUROPSYCHIATRIC INTERVIEW

NEUROPSYCHIATRIC INTERVIEW

Based on screening questions in the MINI-plus

(D. Sheehan, J. Janavs, R. Baker, K. Harnett-Sheehan, E. Knapp, M. Sheehan, University of South Florida, Tampa, USA
and Y. Lecrubier, E. Weiller, T. Hergueta, P. Amorim, L. I. Bonora, J. P. Lépine
Hôpital de la Salpêtrière - Paris)

Date (ddMonyy):

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Instructions:

1. When "YES" investigator and study physician have to discuss the subject before inclusion
2. In the gray fields all items have to be answered "YES".

Depression	NO	YES
Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	<input type="checkbox"/>	<input type="checkbox"/>
In the past two weeks, have you been less interested in most things or less able to enjoy the things you used to enjoy most of the time?	<input type="checkbox"/>	<input type="checkbox"/>
Have you felt sad, low or depressed most of the time for the last two years?	<input type="checkbox"/>	<input type="checkbox"/>
Did you ever make a suicide attempt?	<input type="checkbox"/>	<input type="checkbox"/>

Mania	NO	YES
Have you ever had a period of time when you were feeling 'up' or 'high' or so full of energy or full of yourself that you got into trouble, or that other people thought you were not your usual self? (Do not consider times when you were intoxicated on drugs or alcohol.)	<input type="checkbox"/>	<input type="checkbox"/>
Are you currently feeling 'up' or 'high' or full of energy? IF PATIENT IS PUZZLED OR UNCLEAR ABOUT WHAT YOU MEAN BY 'UP' OR 'HIGH', CLARIFY AS FOLLOWS: By 'up' or 'high' I mean: having elated mood; increased energy; needing less sleep; having rapid thoughts; being full of ideas; having an increase in productivity, motivation, creativity, or impulsive behavior.	<input type="checkbox"/>	<input type="checkbox"/>
Have you ever been persistently irritable, for several days, so that you had arguments or verbal or physical fights, or shouted at people outside your family? Have you or others noticed that you have been more irritable or over reacted, compared to other people, even in situations that you felt were justified?	<input type="checkbox"/>	<input type="checkbox"/>
Are you currently feeling persistently irritable?	<input type="checkbox"/>	<input type="checkbox"/>

Anxiety	NO	YES
Have you, on more than one occasion, had spells or attacks when you suddenly felt anxious, frightened, uncomfortable or uneasy, even in situations where most people would not feel that way?	<input type="checkbox"/>	<input type="checkbox"/>
Did the spells peak within 10 minutes?	<input type="checkbox"/>	<input type="checkbox"/>
Do you feel anxious or uneasy in places or situations where you might have a panic attack or the panic-like symptoms we just spoke about, or where help might not be available or escape might be difficult: like being in a crowd, standing in a line (queue), when you are alone away from home or alone at home, or when crossing a bridge, traveling in a bus, train or car?	<input type="checkbox"/>	<input type="checkbox"/>
In the past month, were you fearful or embarrassed being watched, being the focus of attention, or fearful of being humiliated? This includes things like speaking in public, eating in public or with others, writing while someone watches, or being in social situations.	<input type="checkbox"/>	<input type="checkbox"/>
Have you worried excessively or been anxious about several things over the past 6 months?	<input type="checkbox"/>	<input type="checkbox"/>
Are these worries present most days?	<input type="checkbox"/>	<input type="checkbox"/>

Obsessive compulsive disorder	NO	YES
In the past month, have you been bothered by recurrent thoughts, impulses, or images that were unwanted, distasteful, inappropriate, intrusive, or distressing? (For example, the idea that you were dirty, contaminated or had germs, or fear of contaminating others, or fear of harming someone even though you didn't want to, or fearing you would act on some impulse, or fear or superstitions that you would be responsible for things going wrong, or obsessions with sexual thoughts, images or impulses, or hoarding, collecting, or religious obsessions.)	<input type="checkbox"/>	<input type="checkbox"/>
(DO NOT INCLUDE SIMPLY EXCESSIVE WORRIES ABOUT REAL LIFE PROBLEMS. DO NOT INCLUDE OBSESSIONS DIRECTLY RELATED TO EATING DISORDERS, SEXUAL DEVIATIONS, PATHOLOGICAL GAMBLING, OR ALCOHOL OR DRUG ABUSE BECAUSE THE PATIENT MAY DERIVE PLEASURE FROM THE ACTIVITY AND MAY WANT TO RESIST IT ONLY BECAUSE OF ITS NEGATIVE CONSEQUENCES.)		

NEUROPSYCHIATRIC INTERVIEW *Continued*

PTSD	NO	YES
Have you ever experienced or witnessed or had to deal with an extremely traumatic event that included actual or threatened death or serious injury to you or someone else?	<input type="checkbox"/>	<input type="checkbox"/>
EXAMPLES OF TRAUMATIC EVENTS INCLUDE: SERIOUS ACCIDENTS, SEXUAL OR PHYSICAL ASSAULT, A TERRORIST ATTACK, BEING HELD HOSTAGE, KIDNAPPING, FIRE, DISCOVERING A BODY, SUDDEN DEATH OF SOMEONE CLOSE TO YOU, WAR, OR NATURAL DISASTER.		
Did you respond with intense fear, helplessness or horror?	<input type="checkbox"/>	<input type="checkbox"/>
During the past month, have you re-experienced the event in a distressing way (such as, dreams, intense recollections, flashbacks or physical reactions)?	<input type="checkbox"/>	<input type="checkbox"/>

Addiction	NO	YES
In the past 12 months , have you had 3 or more alcoholic drinks within a 3 hour period on 3 or more occasions?	<input type="checkbox"/>	<input type="checkbox"/>
Have you ever got into trouble by the use of alcohol and/or have you ever been tackled by someone about your drinking behavior?	<input type="checkbox"/>	<input type="checkbox"/>
In the past 12 months , did you take any of these drugs more than once, to get high, to feel better, or to change your mood?	<input type="checkbox"/>	<input type="checkbox"/>
Cross each drug taken:		
Stimulants: <input type="checkbox"/> amphetamine, <input type="checkbox"/> 'speed', <input type="checkbox"/> crystal meth, <input type="checkbox"/> Dexedrine, <input type="checkbox"/> Ritalin, <input type="checkbox"/> diet pills, <input type="checkbox"/> 'Rush'.		
Cocaine: <input type="checkbox"/> snorting, <input type="checkbox"/> IV, <input type="checkbox"/> freebase, <input type="checkbox"/> crack, <input type="checkbox"/> speedball.		
Narcotics: <input type="checkbox"/> heroin, <input type="checkbox"/> morphine, <input type="checkbox"/> opium, <input type="checkbox"/> Dilaudid <input type="checkbox"/> Demerol <input type="checkbox"/> methadone, <input type="checkbox"/> codeine, <input type="checkbox"/> Percodan, <input type="checkbox"/> Darvon.		
Hallucinogens: <input type="checkbox"/> LSD (acid), <input type="checkbox"/> mescaline, <input type="checkbox"/> PCP ('angel dust'), <input type="checkbox"/> 'mushrooms', <input type="checkbox"/> XTC, <input type="checkbox"/> MDA, <input type="checkbox"/> MDMA, <input type="checkbox"/> peyote, <input type="checkbox"/> psilocybin, <input type="checkbox"/> STP		
Inhalants: <input type="checkbox"/> glue, <input type="checkbox"/> ethylchloride, <input type="checkbox"/> laughing gas, <input type="checkbox"/> amyl- of butyl nitraat ('poppers').		
Marijuana: <input type="checkbox"/> hashish (hasj), <input type="checkbox"/> THC, <input type="checkbox"/> weed, <input type="checkbox"/> "pot", <input type="checkbox"/> "grass", <input type="checkbox"/> "reefer".		
Tranquillizers: <input type="checkbox"/> Quaalude <input type="checkbox"/> Seconal ('reds'), <input type="checkbox"/> Valium, <input type="checkbox"/> Xanax, <input type="checkbox"/> Librium, <input type="checkbox"/> Ativan, <input type="checkbox"/> Dalmane, <input type="checkbox"/> Halcion, <input type="checkbox"/> barbiturates <input type="checkbox"/> Miltown.		
Miscellaneous: <input type="checkbox"/> steroids, <input type="checkbox"/> nonprescription sleep or diet pills, <input type="checkbox"/> GHB <input type="checkbox"/> Any others?		

NEUROPSYCHIATRIC INTERVIEW Continued

Does the participant have a family history of panic disorder/panic attacks. (✓)	YES		NO	
Has the interview revealed anything abnormal? (✓)	YES		NO	

If YES, please specify below

Comments	
-----------------	--

<i>Please tick (✓)</i>		YES	NO
Currently healthy			
Age between 18 and 55 inclusive			
BP < 140/90			
HR between 50-90			
BMI between 18 and 28 kg/m ²			
If <i>female</i>	Not pregnant/ breastfeeding		
	Using adequate contraception/ abstinent		
No history of alcohol abuse and negative breath alcohol test			
No history of drug dependence			
Consumes ≤ 8 caffeinated drinks per day			
Does not smoke on a daily basis			
Signed Informed Consent			
Current or History of	Personal psychiatric illness		
	Strong family history of mood disorder, inc. panic disorder		
	Cardiovascular/respiratory disease inc. asthma		
	Epilepsy or Migraine requiring treatment		
	Attacks of hyperventilation		
Current medical illness or clinically significant acute illness within 7 days prior to study start			
Medication in last 8 weeks (apart from local treatment, occasional aspirin or paracetamol, oral, injectable or skin patch contraception, or stable			

OVERALL CRITERIA AND ELIGIBILITY

In the Investigator's opinion, are the overall criteria met? (✓)	YES		NO	
<i>If no, which criteria were not met and why?</i>				
In the Investigator's opinion, on the basis of the screening assessments, is the subject eligible to participate in this study? (✓)	YES		NO	

A.5 CONSENT FORM

(Study ID = 4105: version 1, 19/09/2012)

Study title: **EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON COGNITION & EMOTION I**

Principal Investigator: Dr Matt Garner:
Research Assistants: Dr Daniel Meron, Joanna Miler

Study reference: 4105
Ethics reference: 4105

Please initial the box(es) if you agree with the statement(s):

I have read and completed the information sheet v1 (dated 19/9/12) and have had the opportunity to ask questions about the study

I agree to take part in this research project and agree for my data to be used for the purpose of this study

I understand my participation is voluntary and I may withdraw at any time without my legal rights being affected

Name of participant (print name).....

Signature of participant.....

Date.....

Name of experimenter (print name).....

Signature of experimenter.....

Date.....

A.6 SCALES

STAI

INSTRUCTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and circle the appropriate number to the right of the statement to indicate how you **generally** feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

		Not at all	Somewhat	Moderately so	Very much so
1)	I feel pleasant.....	1	2	3	4
2)	I feel nervous & restless.....	1	2	3	4
3)	I feel satisfied with myself.....	1	2	3	4
4)	I wish I could be as happy as others seem to be.....	1	2	3	4
5)	I feel like a failure	1	2	3	4
6)	I feel rested.....	1	2	3	4
7)	I am 'calm, cool and collected'.....	1	2	3	4
8)	I feel that difficulties are piling up so that I cannot overcome them....	1	2	3	4
9)	I worry too much over something that doesn't really matter.....	1	2	3	4
10)	I am happy.....	1	2	3	4
11)	I have disturbing thoughts.....	1	2	3	4
12)	I lack self-confidence.....	1	2	3	4
13)	I feel secure.....	1	2	3	4
14)	I make decisions easily.....	1	2	3	4
15)	I feel inadequate.....	1	2	3	4
16)	I am content.....	1	2	3	4
17)	Some unimportant thought runs through my mind and bothers me.....	1	2	3	4
18)	I take disappointments so keenly that I can't put them out of my mind.....	1	2	3	4
19)	I am a steady person.....	1	2	3	4
20)	I get in a state of tension or turmoil as I think over my recent concerns and interests.	1	2	3	4

ASI

Circle the one phrase that best represents the extent to which you agree with each item below. If any of the items concern something which is not part of your experience (e.g., "It scares me when I feel shaky" for someone who has never trembled or had the "shakes"), answer on the basis of how you think you might feel if you had such an experience. Otherwise, answer all items on the basis of your own experience.

1. It is important to me not to appear nervous.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

2. When I cannot keep my mind on a task, I worry that I might be going crazy.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

3. It scares me when I feel shaky (trembling).

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

4. It scares me when I feel faint.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

5. It is important to me to stay in control of my emotions.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

6. It scares me when my heart beats rapidly.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

7. It embarrasses me when my stomach growls.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

8. It scares me when I am nauseous.

Very little	A little	Some	Much	Very much
-------------	----------	------	------	-----------

9. When I notice that my heart beats rapidly, I worry that I might have a heart attack..

- | | Very little | A little | Some | Much | Very much |
|-----|--|----------|------|------|-----------|
| 10. | It scares me when I become short of breath. | | | | |
| | Very little | A little | Some | Much | Very much |
| 11. | When my stomach is upset, I worry that I might be seriously ill. | | | | |
| | Very little | A little | Some | Much | Very much |
| 12. | It scares me when I am unable to keep my mind on a task. | | | | |
| | Very little | A little | Some | Much | Very much |
| 13. | Other people notice when I feel shaky. | | | | |
| | Very little | A little | Some | Much | Very much |
| 14. | Unusual body sensations scare me. | | | | |
| | Very little | A little | Some | Much | Very much |
| 15. | When I am nervous, I worry that I might be mentally ill. | | | | |
| | Very little | A little | Some | Much | Very much |
| 16. | It scares me when I am nervous. | | | | |
| | Very little | A little | Some | Much | Very much |

GAD-7

Generally how often have you been bothered by the following problems? Rate each word by drawing a vertical line on the scale below to indicate the extent you have felt that way.

FEELING NERVOUS, ANXIOUS OR ON EDGE

Not at all sure Several days Over half the days Nearly every day

.....

NOT BEING ABLE TO STOP OR CONTROL WORRYING

Not at all sure Several days Over half the days Nearly every day

.....

WORRYING TOO MUCH ABOUT DIFFERENT THINGS

Not at all sure Several days Over half the days Nearly every day

.....

TROUBLE RELAXING

Not at all sure Several days Over half the days Nearly every day

.....

BEING SO RESTLESS THAT IT IS HARD TO SIT STILL

Not at all sure Several days Over half the days Nearly every day

.....

BECOMING EASILY ANNOYED OR IRRITABLE

Not at all sure Several days Over half the days Nearly every day

.....

FEELING AFRAID AS IF SOMETHING AWFUL MIGHT HAPPEN

Not at all sure Several days Over half the days Nearly every day

.....

GAD-7 (BASELINE)

During the last **20 minutes** how often have you been bothered by the following problems? Rate each word by drawing a vertical line on the scale below to indicate the extent you feel this way.

FEELING NERVOUS, ANXIOUS OR ON EDGE

Not at all sure Some of the time Most of the time All of the time

.....

NOT BEING ABLE TO STOP OR CONTROL WORRYING

Not at all sure Some of the time Most of the time All of the time

.....

WORRYING TOO MUCH ABOUT DIFFERENT THINGS

Not at all sure Some of the time Most of the time All of the time

.....

TROUBLE RELAXING

Not at all sure Some of the time Most of the time All of the time

.....

BEING SO RESTLESS THAT IT IS HARD TO SIT STILL

Not at all sure Some of the time Most of the time All of the time

.....

BECOMING EASILY ANNOYED OR IRRITABLE

Not at all sure Some of the time Most of the time All of the time

.....

FEELING AFRAID AS IF SOMETHING AWFUL MIGHT HAPPEN

Not at all sure Some of the time Most of the time All of the time

.....

SSAI BASELINE

INSTRUCTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and circle the appropriate number to the right of the statement to indicate how you **feel** right **now**, that is at **this moment**. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

		Not at all	Somewhat	Moderately so	Very much so
1)	I feel calm.....	1	2	3	4
2)	I feel secure.....	1	2	3	4
3)	I am tense.....	1	2	3	4
4)	I feel strained.....	1	2	3	4
5)	I feel at ease.....	1	2	3	4
6)	I feel upset.....	1	2	3	4
7)	I am presently worrying over possible misfortunes.....	1	2	3	4
8)	I feel satisfied.....	1	2	3	4
9)	I feel frightened.....	1	2	3	4
10)	I feel comfortable.....	1	2	3	4
11)	I feel self-confident.....	1	2	3	4
12)	I feel nervous.....	1	2	3	4
13)	I am jittery.....	1	2	3	4
14)	I feel indecisive.....	1	2	3	4
15)	I am relaxed.....	1	2	3	4
16)	I feel content.....	1	2	3	4
17)	I am worried.....	1	2	3	4
18)	I feel confused.....	1	2	3	4
19)	I feel steady.....	1	2	3	4
20)	I feel pleasant.....	1	2	3	4

PANAS BASELINE

This scale consists of a number of words that describe different feelings and emotions. Read each item and circle the number that indicates to what extent you feel this way **RIGHT NOW**.

	Very slightly or Not at all	A Little	Moderately	Quite a Bit	Extremely
1. interested	1	2	3	4	5
2. distressed	1	2	3	4	5
3. excited	1	2	3	4	5
4. upset	1	2	3	4	5
5. strong	1	2	3	4	5
6. guilty	1	2	3	4	5
7. scared	1	2	3	4	5
8. hostile	1	2	3	4	5
9. enthusiastic	1	2	3	4	5
10. proud	1	2	3	4	5
11. irritable	1	2	3	4	5
12. alert	1	2	3	4	5
13. ashamed	1	2	3	4	5
14. inspired	1	2	3	4	5
15. nervous	1	2	3	4	5
16. determined	1	2	3	4	5
17. attentive	1	2	3	4	5
18. jittery	1	2	3	4	5
19. active	1	2	3	4	5
20. afraid	1	2	3	4	5

VISUAL ANALOGUE SCALE BASELINE (STUDY 1 and 2)

The scale below consists of words describing different feelings and emotions. Rate each word by drawing a vertical line on the scale below to indicate the extent you feel that way **RIGHT NOW**

ANXIOUS

Not at all A Little Moderately Quite a Bit Extremely

.....

ALERT

Not at all A Little Moderately Quite a Bit Extremely

.....

FEEL LIKE LEAVING

Not at all A Little Moderately Quite a Bit Extremely

.....

HAPPY

Not at all A Little Moderately Quite a Bit Extremely

.....

RELAXED

Not at all A Little Moderately Quite a Bit Extremely

.....

WORRIED

Not at all A Little Moderately Quite a Bit Extremely

.....

BASELINE VISUAL ANALOGUE SCALE (STUDY 3)

Put a vertical line at an appropriate point on the line below to indicate **HOW YOU ARE FEELING RIGHT NOW** with regard to that word

ANXIOUS

Not at all A little Moderately Quite a lot Extremely

.....

ALERT

Not at all A little Moderately Quite a lot Extremely

.....

NERVOUS

Not at all A little Moderately Quite a lot Extremely

.....

RELAXED

Not at all A little Moderately Quite a lot Extremely

.....

HAPPY

Not at all A little Moderately Quite a lot Extremely

.....

WORRIED

Not at all A little Moderately Quite a lot Extremely

.....

ATTENTIVE

Not at all A little Moderately Quite a lot Extremely

.....

MINDFUL

Not at all A little Moderately Quite a lot Extremely

.....

VISUAL ANALOGUE SCALE BASELINE (STUDY 4)

ANXIOUS

Not at all A Little Moderately Quite a Bit Extremely

FEARFUL

Not at all A Little Moderately Quite a Bit Extremely

FEEL LIKE LEAVING

Not at all A Little Moderately Quite a Bit Extremely

HAPPY

Not at all A Little Moderately Quite a Bit Extremely

RELAXED

Not at all A Little Moderately Quite a Bit Extremely

WORRIED

Not at all A Little Moderately Quite a Bit Extremely

ALERT

Not at all A Little Moderately Quite a Bit Extremely

ABLE TO CONCENTRATE

Not at all A Little Moderately Quite a Bit Extremely

ACS**Please rate each of these items on a scale of 1 (almost never) to 4 (always).**

1.	It's very hard for me to concentrate on a difficult task when there are noises around.	1	2	3	4
2.	When I need to concentrate and solve a problem, I have trouble focusing my attention.	1	2	3	4
3.	When I am working hard on something, I still get distracted by events around me.	1	2	3	4
4.	My concentration is good even if there is music in the room around me.	1	2	3	4
5.	When concentrating, I can focus my attention so that I become unaware of what's going on in the room around me.	1	2	3	4
6.	When I am reading or studying, I am easily distracted if there are people talking in the same room.	1	2	3	4
7.	When trying to focus my attention on something, I have difficulty blocking out distracting thoughts.	1	2	3	4
8.	I have a hard time concentrating when I am excited about something.	1	2	3	4
9.	When concentrating I ignore feelings of hunger or thirst.	1	2	3	4
10.	I can quickly switch from one task to another.	1	2	3	4
11.	It takes me a while to get really involved in a new task.	1	2	3	4
12.	It is difficult for me to coordinate my attention between the listening and writing required when taking notes during lectures.	1	2	3	4
13.	I can become interested in a new topic very quickly if I need to.	1	2	3	4
14.	It is easy for me to read or write while I'm also talking on the phone.	1	2	3	4
15.	I have trouble carrying on two conversations at once.	1	2	3	4
16.	I have a hard time coming up with new ideas quickly.	1	2	3	4
17.	After being interrupted or distracted, I can easily shift my attention back to what I was doing before.	1	2	3	4
18.	When a distracting thought comes to mind, it is easy for me to shift my attention away from it.	1	2	3	4
19.	It is easy for me to alternate between two different tasks	1	2	3	4
20.	It is hard for me to break from one way of thinking about something and look at it from another point of view.	1	2	3	4

A.7 STUDY ONE: INFORMATION SHEET

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON COGNITION AND EMOTION 1

Research Team: Jo Miler, Verity Pinkney, Dr Dan Meron, (UG student to be confirmed)
Supervisor: Dr Matt Garner

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part and remember that your participation is voluntary.

What is the purpose of the research study?

A recent World Health Organisation study reports that anxiety disorders are more common than many other psychiatric disorders and that because they often result in impairment of daily life, are among the most burdensome of diseases. Generalised Anxiety Disorder (GAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from GAD experience excessive anxiety and worry, and a range of symptoms including muscular tension, restlessness, dizziness, feelings of unreality, difficulty in concentrating, and a feeling of being 'keyed up', often with increases in heart rate, blood pressure and sweating. There are also a number of cognitive biases present in GAD.

In this study we are interested in examining the effects of Transcranial Direct Current Stimulation (tDCS) on individuals' performance in a range of computerised tasks. tDCS is a non-invasive form of brain stimulation, a weak direct current is passed via electrodes into the scalp. This modulates cortical excitability and spontaneous neural activity of underlying brain tissue in accordance with the polarity of stimulation (anodal stimulation increases excitability, whereas cathodal stimulation decreases it). In recent years a growing body of evidence has emerged which supports the efficacy and safety of Left anodal tDCS for the treatment of depression (Kalu UG et al. 2012). To date there are no published studies of tDCS with anxiety as primary outcome measure. Although there are indications that tDCS is associated with reduced anxiety levels, it is still unclear what the optimal tDCS targets and stimulation parameters for the treatment of anxiety are. In this study we are interested in assessing the effects of tDCS on cognitive performance in healthy population.

We plan to recruit healthy male and female volunteers, aged between 18 and 55 years and randomizing them to one of three experimental conditions in which they undergo Left dorsolateral prefrontal cortex anodal tDCS, Right dorsolateral prefrontal cortex anodal tDCS or sham (placebo) tDCS before completing a number of cognitive tasks.

Why have I been chosen?

You have been invited to participate since you have enquired about our advertised studies.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form prior to any further participation. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your future or be held against you in any way.

What does the research study involve?

Before entering the research study the researchers will contact you and ask specific questions about your medical and psychiatric history, to check that you are fit to take part in the study. This will take about 5 minutes, will be arranged at your convenience and can be completed over the phone if helpful. All information will remain confidential.

You should be generally healthy and have no history of epilepsy or migraine. Your alcohol intake should not be more than 50 units per week (male) or 35 units per week (female). Females should not be pregnant or breast feeding, or considering becoming pregnant. You should not have electronic or metal implants. Your eligibility according to the above will be determined over the course of the telephone screening (5 min) and pre-test interview (10 min).

If you are found to meet our list of entry criteria, then you will be invited to complete the study.

What do I have to do on the study day?

YOUR TIME COMMITMENT IN THE RESEARCH STUDY IS AS FOLLOWS:

You will be required to attend one test session of approximately 1.5 hour at the Psychology Department, University of Southampton. You will remain seated in a comfortable position throughout the testing session.

You will undergo 20 minutes of transcranial direct current stimulation (tDCS). The procedure involves placing two skin surface electrodes encased in a sponge which has been soaked in normal saline (water and salt solution), one electrode on the left of your forehead and the other on the right. The electrodes will deliver a low-intensity direct current (1-2mA) from a tDCS stimulator. After the tDCS session you will be asked about any thoughts that occurred during the stimulation period, will complete a number of short computerized tasks and will be asked to complete a 15 minute thought sampling task in which you will be asked to focus your attention on your breathing and a current worry/concern. Please remember that you are free to withdraw from the study at any time. You will also be asked to complete some questionnaires to measure how you are feeling and have your blood pressure taken using a standard arm-cuff monitor.

What are the side effects of the tDCS treatments?

During tDCS and / or after the session, you may experience a headache. tDCS can also be associated with skin irritation, itchiness, discomfort and/or burning sensation at the site of stimulation. Very rarely, people have reported elevation of mood (becoming somewhat 'high' in mood) following tDCS, but this is not likely unless there is a history of mood disorders (such as depression or bipolar disorder). tDCS could interfere with the functioning of electronic implants and should also not be undertaken if you have metal implants of any kind.

The researchers will remain near you at all times and will offer reassurance if necessary. If you feel uncomfortable at any time during the procedure you may indicate that you wish the procedure to stop.

What are the possible disadvantages and risks of taking part?

Pregnant women should not take part in this study, and neither should women who plan to become pregnant. All women will therefore be asked to answer some short questions about their use of contraception to exclude the possibility of pregnancy.

What are the possible benefits of taking part?

You will not expect to directly benefit from taking part in this research study and your participation is voluntary. However, the information we get from this study may help us to understand the cognitive benefits of tDCS and thus support its use to treat patients with anxiety disorders in the future.

What if new information becomes available?

We do not expect any information about the effects of the tDCS procedure to become available, but if this happens this information will be passed on to you immediately.

Will my taking part in this study be kept confidential?

All data is anonymized and confidential. You will be assigned a unique participant number that will be used to identify your data – your name will not be linked with or stored with any of your data. Any information and research study documentation taken for this research study will remain confidential and will be available only to the principal investigator and members of his research team directly involved in the project.

What will happen to the results of the research study?

When the study has been completed, we shall analyse the data and report the findings. This will be reported in an appropriate scientific journal or presented at a scientific meeting. You would not be identified in any way and if you would like a copy of the final paper, you may request this.

Who is organising and funding the research?

The study is being organised and sponsored by the University of Southampton.

Who has reviewed the study?

The study has received approval from an appropriate ethics panel within the University of Southampton, and the University of Southampton Research Governance Office.

Who can I contact for further information?

For further queries, please contact Dr Matt Garner.

Dr Matt Garner
m.j.garner@soton.ac.uk
02380825539

If you participate in this study you will be given a copy of this information sheet

A.8 STUDY ONE: DEBRIEF

Debrief (Version 1.) 19/09/2012 Study ID: 4105

EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON COGNITION AND EMOTION 1

Thank you for taking part in our experiment.

Study background: transcranial direct current stimulation (tDCS) is a novel treatment modality with a growing evidence base for efficacy and safety in the treatment of depressive disorders. We are very interested to explore its potential for treating anxiety disorders. To date there are no published studies of tDCS in anxiety disorders although evidence is emerging which suggests that tDCS may be useful in reducing anxiety symptoms.

There are a number of cognitive biases present in anxiety disorders. This study explores whether or not a session of transcranial direct current stimulation (tDCS) modifies some of the biases in cognition and emotion processing that confer risk for anxiety disorders such as Generalised Anxiety Disorder (GAD). You were randomised to receive either a 20 minute session of Left sided anodal tDCS, of Right sided anodal tDCS or of sham (placebo) tDCS prior to completing a number of computerised task including that measured various aspects of your attention, and processing of positive and negative emotional information.

If you are interested in finding out more about the rationale behind this research and methods used in this project please see the references below:

1. U. G. Kalu, C. E. Sexton, C. K. Loo and K. P. Ebmeier Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. *Psychological Medicine*, Available on CJO 2012 doi:10.1017/S0033291711003059
2. Brunoni, A.R., Fregni, F., (2011) Clinical trial design in non-invasive brain stimulation psychiatric research. *International Journal of Methods in Psychiatric Research*. 20(2): e19–e30, June 2011

During this study we have asked you to reflect on certain aspects of your physical health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner.

As noted in the information sheet – individuals vary in their response to a session of tDCS with most individuals feeling completely normal by the end of the experimental session. Some participants may experience skin irritation, tingling, itching or burning sensation at tDCS stimulation site during and/or after stimulation. In the unlikely event that you feel unwell please contact your General Practitioner or NHS Direct on 08454647 as per usual.

Finally, if you have more general worries during your time as a student in Southampton then please also be aware that Student Services or your personal tutor are happy to provide support and advice.

Furthermore if you have any concerns or queries regarding any aspect of your participation in this study then please feel free to contact Dr Matt Garner on m.j.garner@soton.ac.uk or 02380595926 and he will be happy to discuss these with you.

A member of the research team will phone you tomorrow to check that you have not experienced any adverse events following your participation, and to discuss any additional queries that you might have.

Thank you for your participation in this research.

Signature _____ Date _____

Name

If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the chair of the Ethics Committee, Psychology, University of Southampton, SO17 1BJ, UK. Phone: +44 (0)23 8059 4663, email slb1n10@soton.ac.uk

A.9 STUDY TWO: INFORMATION SHEET

Study ID = 2238: version 1, 10/2/2012

INFORMATION FOR PARTICIPANTS

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON SUBJECTIVE, AUTONOMIC AND NEUROPSYCHOLOGICAL RESPONSE TO CARBON DIOXIDE CHALLENGE

Principal Investigator: Dr Matt Garner

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part and remember that your participation is voluntary.

What is the purpose of the research study?

A recent World Health Organisation study reports that anxiety disorders are more common than many other psychiatric disorders and that because they often result in impairment of daily life, are among the most burdensome of diseases. Generalised Anxiety Disorder (GAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from GAD experience excessive anxiety and worry, and a range of symptoms including muscular tension, restlessness, dizziness, feelings of unreality, difficulty in concentrating, and a feeling of being 'keyed up', often with increases in heart rate, blood pressure and sweating.

If there was a human model of GAD, (that is, a way of temporarily producing some of the symptoms of GAD, but in a healthy person) it could be used to discover new and more effective treatments and help us to understand what is happening in the body. To be effective, any potential model would need to reliably produce anxiety in healthy people and the degree of anxiety should be repeatable and measurable.

We have been working on the development of a model of GAD using the inhalation of 7.5% carbon dioxide (CO₂) for 20 minutes. In healthy volunteers this makes some people feel anxious and tense and reduces feelings of being relaxed and happy. It also increases blood pressure and heart rate measures. All the effects of CO₂ are different from the inhalation of normal room air and we believe that this model could be used to explore how and whether treatments work in GAD.

So that we can further investigate the 7.5% CO₂ model of GAD, we examine variables that might alter individuals' response experience of CO₂ inhalation.

In this study we are interested in examining the effects of Transcranial Direct Current Stimulation (tDCS) on individuals' experience of CO₂ inhalation. tDCS is a non-invasive form of brain stimulation, a weak direct current is passed via electrodes into the scalp. This modulates cortical excitability and spontaneous neural activity of underlying brain tissue in accordance with the polarity of stimulation (anodal stimulation increases excitability, whereas cathodal stimulation decreases it). In recent years a growing body of evidence has emerged which supports the efficacy and safety of Left anodal tDCS for the treatment of depression (Kalu UG et al. 2012). To date there are no published studies of tDCS with anxiety as primary outcome measure. Although there are indications that tDCS is associated with reduced anxiety levels, it is still unclear what the optimal tDCS targets and stimulation parameters for the treatment of anxiety are.

We plan to study this by recruiting healthy male and female volunteers, aged between 18 and 55 years and randomizing them to one of three experimental conditions in which they undergo Left dorsolateral prefrontal cortex anodal tDCS, Right dorsolateral prefrontal cortex anodal tDCS or sham (placebo) tDCS before completing the CO₂ inhalation.

Why have I been chosen?

You have been invited to participate since you have enquired about our advertised studies.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form prior to any further participation. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your future or be held against you in any way.

What does the research study involve?

Before entering the research study the researchers will contact you and ask specific questions about your medical and psychiatric history, to check that you are fit to take part in the study. This will take about 10 minutes, will be arranged at your convenience and can be completed over the phone if helpful. All information will remain confidential.

You should be generally healthy, have no history of or current asthma, epilepsy or migraine, and have no present or past anxiety disorder or other mental health problem. You should not take part if a close member of your family suffers from regular panic attacks or has been diagnosed with panic disorder. Your alcohol intake should not be more than 50 units per week (male) or 35 units per week (female). You should not be a regular smoker. No other medication should have been used in the preceding 8 weeks, apart from occasional aspirin or paracetamol, or local treatments. Females should be using adequate methods of contraception and should not be pregnant or breast feeding, or considering becoming pregnant. You should not have electronic or metal implants. Your eligibility according to the above will be determined over the course of the telephone screening (10 min) and pre-test interview (10 min).

If you are found to meet our list of entry criteria, then you will be invited to complete the study.

What do I have to do on the study day?

YOUR TIME COMMITMENT IN THE RESEARCH STUDY IS AS FOLLOWS:

Prior to the testing session you should refrain from alcohol for 36 hours. You should not drink any caffeinated drinks after midnight prior to the day of testing. This is because alcohol and caffeine have effects of their own on blood pressure and heart rate measurements and alcohol may enhance the effects of the gas. However, exception from this is if you regularly ingest caffeine in the

morning. If this is the case, you should have your usual caffeinated drink to avoid withdrawal effects during the study. You should not be a regular (i.e., daily) smoker and should not have smoked within 12 hours of the study session.

You will be required to attend one test session of approximately 3 hours. You will remain seated in a comfortable position throughout the testing session. During the testing session, measurements of your blood pressure and heart rate will be periodically taken using six peripheral (i.e. non-invasive) skin-sensors. You will complete some questionnaires to measure how you are feeling.

Measurements will be taken shortly after you arrive and once you are comfortable. You will undergo 20 minutes of transcranial direct current stimulation (tDCS). The procedure involves placing two skin surface electrodes encased in a sponge which has been soaked in normal saline (water and salt solution), one electrode on the left of your forehead and the other on the right. The electrodes will deliver a low-intensity direct current (1-2mA) from a tDCS stimulator. During the test session you will undergo an inhalation procedure of 20 minutes duration. The physiological measures (i.e., heart rate, blood pressure) and questionnaire measurements will be taken before and after inhalation. During the inhalation procedure you will complete a short computerized task that requires you to press buttons as quickly and accurately as you can. In each task we will place a skin surface electrode on your left and right temple to measure your eye-muscle activity, and will place two skin surface electrodes on the left, and right-hand side of your scalp to record electrical activity in your left and right hemispheres. In one of the tasks you will be presented with a set of widely used experimental emotional pictures, some of which you may find unpleasant. Before you agree to participate you will have the opportunity view example emotional pictures. Please remember that you are free to withdraw from the study at anytime.

The 20 minute gas inhalation will be administered through a mask, which covers your mouth and nose. This will be fitted prior to inhalation of the gas to enable you to become accustomed to wearing it. You will then wear the mask during the inhalation.

Any effects of the gas inhalation are temporary, and at the end of the study session you will remain in the testing room until you feel that any effects of the gas have worn off. We will contact you the day after the study day to check that you are healthy and well.

What are the side effects of the tDCS treatments?

It is important to stress that the tDCS equipment we will use is designed to be used by patients with depression at their home without supervision. We will be using it in a controlled laboratory environment under constant supervision.

During tDCS and / or after the session, you may experience a headache. tDCS can also be associated with skin irritation, itchiness, discomfort and/or burning sensation at the site of stimulation. Very rarely, people have reported elevation of mood (becoming somewhat 'high' in mood) following tDCS, but this is not likely unless there is a history of mood disorders (such as depression or bipolar disorder). tDCS could interfere with the functioning of electronic implants and should also not be undertaken if you have metal implants of any kind.

What are the gas mixtures being delivered?

The 7.5% CO₂ gas is a mixture of carbon dioxide and air, with the air containing the usual amount of oxygen. The air will be normal air that is administered via a mask in the same way as the CO₂.

What are the side effects of the gas treatments?

Carbon dioxide inhalation may cause feelings of anxiety or unpleasantness. Other physiological effects that may occur include racing of heart, dizziness, pins and needles, and breathlessness. Some people also experience a mild headache afterwards.

People experience and describe the effects of inhaling 7.5% CO₂ gas in different ways, and there is

no way of knowing in advance how you will respond. Some people do not notice it at all, and some experience more marked anxiety. Most people will notice some effects, and if you do not like the effects you can ask to stop. These feelings should be short-lived (resolve within 5 minutes) and will not cause any lasting harm.

The researchers will remain near you at all times and will offer reassurance if necessary. If you feel uncomfortable breathing the gas at any time during the procedure you may indicate that you wish the procedure to stop.

What are the possible disadvantages and risks of taking part?

Pregnant women should not take part in this study, and neither should women who plan to become pregnant. All women will therefore be asked to answer 6 short questions about their use of contraception to exclude the possibility of pregnancy.

What are the possible benefits of taking part?

You will not expect to directly benefit from taking part in this research study and your participation is voluntary. However, the information we get from this study may help us to understand and treat patients with anxiety disorders in the future.

What if new information becomes available?

We do not expect any information about the effects of the inhalation procedure to become available, but if this happens this information will be passed on to you immediately.

Will my taking part in this study be kept confidential?

All data is anonymized and confidential. You will be assigned a unique participant number that will be used to identify your data – your name will not be linked with or stored with any of your data. Any information and research study documentation taken for this research study will remain confidential and will be available only to the principal investigator and members of his research team directly involved in the project.

What will happen to the results of the research study?

When the study has been completed, we shall analyse the data and report the findings. This will be reported in an appropriate scientific journal or presented at a scientific meeting. You would not be identified in any way and if you would like a copy of the final paper, you may request this.

Who is organising and funding the research?

The study is being organised and sponsored by the University of Southampton.

Who has reviewed the study?

The study has received approval from an appropriate ethics panel within the University of Southampton, and the University of Southampton Research Governance Office.

Who can I contact for further information?

For further queries, please contact Dr Matt Garner or Prof David Baldwin (see below).

Dr Matt Garner

Prof David Baldwin

m.j.garner@soton.ac.uk

dsb1@soton.ac.uk

02380825539

If you participate in this study you will be given a copy of this information sheet and a signed consent form to keep.

A.10 STUDY TWO: DEBRIEF

Debrief (Version 1.1) 16/2/2012 Study ID: 2238

EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS) ON SUBJECTIVE, AUTONOMIC AND NEUROPSYCHOLOGICAL RESPONSE TO CARBON DIOXIDE CHALLENGE

Thank you for taking part in our experiment.

Study background: Transcranial direct current stimulation (tDCS) is a novel treatment modality with a growing evidence base for efficacy and safety in the treatment of depressive disorders. We are very interested to explore it's potential for treating anxiety disorders. To date there are no published studies of tDCS in anxiety disorders although evidence is emerging which suggests that tDCS may be useful in reducing anxiety symptoms.

Inhalation of 7.5% CO₂ increases anxiety and autonomic arousal in humans and provides a novel experimental model of anxiety in healthy humans. This model is well placed to evaluate promising treatments for anxiety, including psychological treatments and pharmacological drug treatments.

Our study examined the extent to which a session of transcranial direct current stimulation (tDCS) offered protection against CO₂-increased anxiety and autonomic arousal (e.g. heart rate, blood pressure, skin conductance). You were randomized to receive either a 20 minute session of Left sided anodal tDCS, of Right sided anodal tDCS or of sham (placebo) tDCS.

If you are interested in finding out more about the rationale behind this research and methods used in this project please see the references below:

3. Bailey, J. E., Argyropoulos, S. V., Kendrick, A. H., & Nutt, D. J. (2005). Behavioral and cardiovascular effects of 7.5% CO₂ in human volunteers. *Depression and Anxiety*, 21(1), 18-25.
4. Ziemann, A. E., Allen, J. E., Dahdaleh, N. S., Drebot, II, Coryell, M. W., Wunsch, A. M., et al. (2009). The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell*, 139(5), 1012-1021.
5. Garner, M., A. Attwood, et al. (2011). Inhalation of 7.5% Carbon Dioxide Increases Threat Processing in Humans. *Neuropsychopharmacology* 36(8): 1557-1562.
6. U. G. Kalu, C. E. Sexton, C. K. Loo and K. P. Ebmeier Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. *Psychological Medicine*, Available on CJO 2012 doi:10.1017/S0033291711003059
7. Brunoni, A.R., Fregni, F., (2011) Clinical trial design in non-invasive brain stimulation psychiatric research. *International Journal of Methods in Psychiatric Research*. 20(2): e19–e30, June 2011

During this study we have asked you to reflect on certain aspects of your physical and mental health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner.

As noted in the information sheet – individuals vary in their response to the inhalation of CO₂ and to a session of tDCS with most individuals feeling completely normal by the end of the experimental session. Occasionally individuals report a mild headache for a short time afterwards. Some participants may experience skin irritation, tingling, itching or

burning sensation at tDCS stimulation site during and/or after stimulation. In the unlikely event that you feel unwell please contact your General Practitioner or NHS Direct on 08454647 as per usual.

Finally, if you have more general worries during your time as a student in Southampton then please also be aware that Student Services or your personal tutor are happy to provide support and advice.

Furthermore if you have any concerns or queries regarding any aspect of your participation in this study then please feel free to contact Dr Matt Garner on m.j.garner@soton.ac.uk or 02380595926 and he will be happy to discuss these with you.

A member of the research team will phone you tomorrow to check that you have not experienced any adverse events following your participation, and to discuss any additional queries that you might have.

Thank you for your participation in this research.

Signature _____ Date _____

Name

If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the Chair of the Ethics Committee, Psychology, University of Southampton, Southampton, SO17 1BJ. Phone: (023) 8059 5578.

A.11 STUDY THREE: INFO SHEET

Study ID = 742: version 1, 4/10/11

INFORMATION FOR PARTICIPANTS

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

EFFECTS OF ATTENTION TRAINING ON SUBJECTIVE, AUTONOMIC AND NEUROPSYCHOLOGICAL RESPONSE TO CARBON DIOXIDE CHALLENGE

Principal Investigator: Dr Matt Garner

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part and remember that your participation is voluntary.

What is the purpose of the research study?

A recent World Health Organisation study reports that anxiety disorders are more common than many other psychiatric disorders and that because they often result in impairment of daily life, are among the most burdensome of diseases. Generalised Anxiety Disorder (GAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from GAD experience excessive anxiety and worry, and a range of symptoms including muscular tension, restlessness, dizziness, feelings of unreality, difficulty in concentrating, and a feeling of being 'keyed up', often with increases in heart rate, blood pressure and sweating.

If there was a human model of GAD, (that is, a way of temporarily producing some of the symptoms of GAD, but in a healthy person) it could be used to discover new and more effective treatments and help us to understand what is happening in the body. To be effective, any potential model would need to reliably produce anxiety in healthy people and the degree of anxiety should be repeatable and measurable.

We have been working on the development of a model of GAD using the inhalation of 7.5% carbon dioxide (CO₂) for 20 minutes. In healthy volunteers this makes some people feel

anxious and tense and reduces feelings of being relaxed and happy. It also increases blood pressure and heart rate measures. All the effects of CO₂ are different from the inhalation of normal room air and we believe that this model could be used to explore how and whether treatments work in GAD.

So that we can further investigate the 7.5% CO₂ model of GAD, we examine variables that might alter individuals' response experience of CO₂ inhalation. We plan to do this by recruiting healthy male and female volunteers, aged between 18 and 55 years and randomizing them to one of four experimental conditions in which they complete different tasks before completing the CO₂ inhalation.

Why have I been chosen?

You have been invited to participate since you have enquired about our advertised studies.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form prior to any further participation. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your future or be held against you in any way.

What does the research study involve?

Before entering the research study the researchers will contact you and ask specific questions about your medical and psychiatric history, to check that you are fit to take part in the study. This will take about 10 minutes, will be arranged at your convenience and can be completed over the phone if helpful. All information will remain confidential.

You should be generally healthy, have no history of or current asthma or migraine, and have no present or past anxiety disorder or other mental health problem. You should not take part if a close member of your family suffers from regular panic attacks or has been diagnosed with panic disorder. Your alcohol intake should not be more than 50 units per week (male) or 35 units per week (female). You should not be a regular smoker. No other medication should have been used in the preceding 8 weeks, apart from occasional aspirin or paracetamol, or local treatments. Females should be using adequate methods of contraception and should not be pregnant or breast feeding, or considering becoming pregnant. Your eligibility according to the above will be determined over the course of the telephone screening (10 min) and pre-test interview (10 min).

If you are found to meet our list of entry criteria, then you will be invited to complete the study.

What do I have to do on the study day?

YOUR TIME COMMITMENT IN THE RESEARCH STUDY IS AS FOLLOWS:

Prior to the testing session you should refrain from alcohol for 36 hours. You should not drink any caffeinated drinks after midnight prior to the day of testing. This is because alcohol and caffeine have effects of their own on blood pressure and heart rate measurements and alcohol may enhance the effects of the gas. However, exception from this is if you regularly ingest caffeine in the morning. If this is the case, you should have your usual caffeinated drink to avoid withdrawal effects during the study. You should not be a regular (i.e., daily) smoker and should not have smoked within 12 hours of the study session.

You will be required to attend one test session of approximately 3 hours. You will remain seated in a comfortable position throughout the testing session. During the testing session, measurements of your blood pressure and heart rate will be periodically taken using six peripheral (i.e. non-invasive) skin-sensors. You will complete some questionnaires to measure how you are feeling.

Measurements will be taken shortly after you arrive and once you are comfortable. During the test session you will undergo two inhalation procedures of 20 minutes duration each. The physiological measures (i.e., heart rate, blood pressure) and questionnaire measurements will be taken before and after each inhalation, and there will be a rest period between inhalations. During the two inhalation procedures you will complete a short computerized task that requires you to press buttons as quickly and accurately as you can. In each task we will place a skin surface electrode on your left and right temple to measure your eye-muscle activity, and will place two skin surface electrodes on the left, and right-hand side of your scalp to record electrical activity in your left and right hemispheres. In one of the tasks you will be presented with a set of widely used experimental emotional pictures, some of which you may find unpleasant. Before you agree to participate you will have the opportunity view example emotional pictures. Please remember that you are free to withdraw from the study at anytime.

The 20 minute gas inhalations will be administered through a mask, which covers your mouth and nose. This will be fitted prior to inhalation of the gas to enable you to become accustomed to wearing it. You will then wear the mask during the inhalation.

Any effects of the gas inhalation are temporary, and at the end of the study session you will remain in the testing room until you feel that any effects of the gas have worn off. We will contact you the day after the study day to check that you are healthy and well.

What are the gas mixtures being delivered?

The 7.5% CO₂ gas is a mixture of carbon dioxide and air, with the air containing the usual

amount of oxygen. The air will be normal air that is administered via a mask in the same way as the CO₂.

What are the side effects of the gas treatments?

Carbon dioxide inhalation may cause feelings of anxiety or unpleasantness. Other physiological effects that may occur include racing of heart, dizziness, pins and needles, and breathlessness. Some people also experience a mild headache afterwards.

People experience and describe the effects of inhaling 7.5% CO₂ gas in different ways, and there is no way of knowing in advance how you will respond. Some people do not notice it at all, and some experience more marked anxiety. Most people will notice some effects, and if you do not like the effects you can ask to stop. These feelings should be short-lived (resolve within 5 minutes) and will not cause any lasting harm.

The researchers will remain near you at all times and will offer reassurance if necessary. If you feel uncomfortable breathing the gas at any time during the procedure you may indicate that you wish the procedure to stop.

What are the possible disadvantages and risks of taking part?

Pregnant women should not take part in this study, and neither should women who plan to become pregnant. All women will therefore be asked to answer 6 short questions about their use of contraception to exclude the possibility of pregnancy.

What are the possible benefits of taking part?

You will not expect to directly benefit from taking part in this research study and your participation is voluntary. However, the information we get from this study may help us to understand and treat patients with anxiety disorders in the future.

What if new information becomes available?

We do not expect any information about the effects of the inhalation procedure to become available, but if this happens this information will be passed on to you immediately.

Will my taking part in this study be kept confidential?

All data is anonymized and confidential. You will be assigned a unique participant number that will be used to identify your data – your name will not be linked with or stored with any of your data. Any information and research study documentation taken for this research study will remain confidential and will be available only to the principal investigator and members of his research team directly involved in the project.

What will happen to the results of the research study?

When the study has been completed, we shall analyse the data and report the findings. This will be reported in an appropriate scientific journal or presented at a scientific meeting. You would not be identified in any way and if you would like a copy of the final paper, you may request this.

Who is organising and funding the research?

The study is being organised and sponsored by the University of Southampton.

Who has reviewed the study?

The study has received approval from an appropriate ethics panel within the University of Southampton, and the University of Southampton Research Governance Office.

Who can I contact for further information?

For further queries, please contact Dr Matt Garner or Prof David Baldwin (see below).

Dr Matt Garner

Prof David Baldwin

m.j.garner@soton.ac.uk

dsb1@soton.ac.uk

02380825539

If you participate in this study you will be given a copy of this information sheet and a signed consent form to keep.

A.12 STUDY THREE: DEBRIEF

Debrief (Version 1.0) 4/10/11 Study ID: 742

EFFECTS OF ATTENTION TRAINING ON SUBJECTIVE, AUTONOMIC AND NEUROPSYCHOLOGICAL RESPONSE TO CARBON DIOXIDE CHALLENGE

Thank you for taking part in our experiment.

Study background: Inhalation of 7.5% CO₂ increases anxiety and autonomic arousal in humans and provides a novel experimental model of anxiety in healthy humans. This model is well placed to evaluate promising treatments for anxiety, including psychological treatments and pharmacological drug treatments.

Our study examined the extent to which different psychological interventions offered protection against CO₂-increased anxiety and autonomic arousal (e.g. heart rate, blood pressure, skin conductance). You were randomized to one of several psychological intervention groups: 1) attentional bias modification (in which your attention was trained away from threat stimuli), 2) narrow focus mindfulness training, 3) open-focussed acceptance/mindful training, or control (no intervention).

If you are interested in finding out more about the rationale behind this research and methods used in this project please see the references below:

1. Bailey, J. E., Argyropoulos, S. V., Kendrick, A. H., & Nutt, D. J. (2005). Behavioral and cardiovascular effects of 7.5% CO₂ in human volunteers. *Depression and Anxiety*, 21(1), 18-25.
2. Ziemann, A. E., Allen, J. E., Dahdaleh, N. S., Drebot, II, Coryell, M. W., Wunsch, A. M., et al. (2009). The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell*, 139(5), 1012-1021.
3. Garner, M., A. Attwood, et al. (2011). Inhalation of 7.5% Carbon Dioxide Increases Threat Processing in Humans. *Neuropsychopharmacology* 36(8): 1557-1562.

During this study we have asked you to reflect on certain aspects of your physical and mental health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner.

As noted in the information sheet – individuals vary in their response to the inhalation of CO₂ with most individuals feeling completely normal by the end of the experimental session. Occasionally individuals report a mild headache for a short time afterwards. In the unlikely event that you feel unwell please contact your General Practitioner or NHS Direct on 08454647 as per usual.

Finally, if you have more general worries during your time as a student in Southampton then please also be aware that Student Services or your personal tutor are happy to provide support and advice.

Furthermore if you have any concerns or queries regarding any aspect of your participation in this study then please feel free to contact Dr Matt Garner on m.j.garner@soton.ac.uk or 02380595926 and he will be happy to discuss these with you.

A member of the research team will phone you tomorrow to check that you have not experienced any adverse events following your participation, and to discuss any additional queries that you might have.

Thank you for your participation in this research.

Signature _____ Date _____

Name _____

If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the Chair of the Ethics Committee, Psychology, University of Southampton, Southampton, SO17 1BJ. Phone: (023) 8059 5578.

A.13 STUDY FOUR: INFO SHEET

INFORMATION FOR PARTICIPANTS

EEG brain activity during 7.5% Carbon Dioxide Inhalation in healthy humans

Researcher: Drew Maxfield (MSc student)

Joanna Miler (PhD Student)

Project Supervisor: Dr Matt Garner

ERGO Study ID number: 5444

Please read this information carefully before deciding to take part in this research. If you are happy to participate you will be asked to sign a consent form.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part and remember that your participation is voluntary.

What is the purpose of the research study?

Anxiety disorders are more common than many other psychiatric disorders and because they typically result in impairment of daily life, are among the most burdensome of medical conditions. Generalised Anxiety Disorder (GAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from GAD experience excessive anxiety and worry, and a range of symptoms including muscular tension, restlessness, dizziness, feelings of unreality, difficulty in concentrating, and a feeling of being 'keyed up', often with increases in heart rate, blood pressure and sweating.

If there was a human model of GAD, (that is, a way of temporarily producing some of the symptoms of GAD, but in a healthy person) it could be used to investigate what areas of the brain become active in response to excessive anxiety and worry. To be effective, any potential model would need to reliably produce anxiety in healthy people and the degree of anxiety should be repeatable and measurable.

Past research has shown that inhaling 7.5% carbon dioxide (CO₂) for 20 minutes in human volunteers makes some people feel anxious and tense and reduces feelings of being relaxed and happy. It also temporarily increases blood pressure and heart rate as well as alters attention and emotion processing, which is commonly reported in anxious patients. The effects of CO₂ are different from the inhalation of normal room air and we believe that this model could be used to explore what areas of the brain become active in GAD.

This study will examine how patterns of brain activity respond to CO₂ inhalation in healthy volunteers compared to receiving normal air.

We are recruiting healthy male and female volunteers, aged between 18 and 55 years, to attend a single test session to have their brain activity recorded using Electroencephalography (EEG). This will involve participants having small electrodes placed across their scalp which record the brains electrical signals (given off by groups of millions of neurons) which generate output in the form of brain waves. While wearing these electrodes, participants will complete two inhalation procedures, i) a safe gas mixture of air enriched with 7.5% CO₂ (for 20 minutes), and ii) normal air (also for twenty minutes). Participants will also

complete a 15 minute task in which they will be asked to focus on their breathing for 5 minutes, a current concern for 5 minutes and their breathing again for 5 minutes. Participants will also be asked to complete some questionnaire measures.

Why have I been chosen?

You have been invited to participate since you have enquired about our advertised studies.

Do I have to take part?

No, it is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form prior to further participation. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your future or be held against you in any way.

What does the research study involve?

The research involves attending a 2.5 hour single test session. There are three main parts to the study:

10 minute screening interview/phone-call.

Before entering the research study the researchers will ask specific questions about your medical and psychiatric history, to check that you are fit to take part in the study. This will take about 10 minutes which will be arranged at your convenience and can be completed either over the phone or in person. All information will remain confidential.

You should be generally healthy, have no history of or current asthma, migraine, seizures, renal or hepatic impairment or glaucoma, and have no present or past anxiety disorder or other mental health problem. You should not take part if a close member of your family suffers from regular panic attacks or has been diagnosed with panic disorder. Your alcohol intake should not be more than recommended guidelines; 28 units per week (males), or 21 units per week (female). You should not be a regular smoker. No other medication should have been used in the preceding 8 weeks, apart from occasional aspirin or paracetamol, or local treatments. Females should be using adequate methods of contraception and should not be pregnant or breast feeding, or be considering becoming pregnant. Your eligibility according to the above will be determined over the course of an initial screening session (10 min) conducted on the study day. If you are found to meet our list of entry criteria, then you will be invited to complete the study.

Attaching the EEG electrodes

On confirming your eligibility, you will then have the electrodes attached to your scalp which should take around 40 minutes. These electrodes are completely harmless and do not cause any pain or discomfort. However, we use a small amount of gel in each electrode to aid measurement of the brain's electrical activity, This can make your hair quite messy, especially if you have long hair, and it may take one or two thorough shampoos to remove. It is therefore advised that you do not take part in the study if you have somewhere important to be once the testing session is over, e.g. job interview etc. Washing and drying hair facilities are available to those who wish to take part and want to remove the gel right away after the testing session is complete. Time to wash and dry your hair is included in the experiment and you receive credits for this portion of time.

7.5% CO₂ or normal air inhalation test session.

You will be required to then complete a single gas inhalation session where you will be required to inhale either normal air enriched with 7.5% of CO₂ or normal air for 20 minutes through a nasal face mask. You will remain seated in a comfortable position throughout the testing session. During the testing session,

measurements of your blood pressure and heart rate will be periodically taken using six peripheral (i.e. non-invasive) skin-sensors. You will complete some questionnaires to measure how you are feeling.

Prior to the testing session you should refrain from alcohol for 36 hours. You should not drink any caffeinated drinks after midnight prior to the day of testing. This is because alcohol and caffeine have effects of their own on blood pressure and heart rate measurements and alcohol may enhance the effects of the gas.

However, the exception from this is if you regularly ingest caffeine in the morning. If this is the case, you should have your usual caffeinated drink to avoid withdrawal effects during the study. You should not be a regular (i.e., daily) smoker and should not have smoked within 12 hours of the study session.

Measurements will be taken shortly after you arrive and once you are comfortable. The physiological measures (i.e., heart rate, blood pressure) and questionnaire measurements will be taken before and after inhalation. After the inhalation period you will be asked about any thoughts that occurred during the inhalation. Please remember that you are free to withdraw from the study at any time.

The 20 minute gas inhalation will be administered through a mask, which covers your mouth and nose. This will be fitted prior to inhalation of the gas to enable you to become accustomed to wearing it. You will then wear the mask during the inhalation.

Any effects of the gas inhalation are temporary and typically resolve within a minute after inhalation. At the end of the study session you will remain in the testing room until you feel that any effects of the gas have worn off. We will contact you the day after the study day to check that you are healthy and well.

What are the gas mixtures being delivered?

The 7.5% CO₂ gas is a mixture of carbon dioxide and air, with the air containing the usual amount of oxygen. The air will be normal air that is administered via a mask in the same way as the CO₂ inhalation.

What are the side effects of the gas treatments?

Carbon dioxide inhalation may cause feelings of anxiety or unpleasantness. Other physiological effects that may occur include racing of heart, dizziness, pins and needles, and breathlessness. Some people also experience a mild headache afterwards.

People experience and describe the effects of inhaling 7.5% CO₂ gas in different ways, and there is no way of knowing in advance how you will respond. Some people do not notice it at all, and some experience more marked anxiety. Most people will notice some effects, and if you do not like the effects you can ask to stop. These feelings should be short-lived (typically resolving within a couple of minutes) and would not cause any lasting harm.

The researchers will remain near you at all times and will offer reassurance if necessary. If you feel uncomfortable breathing the gas at any time during the procedure you may indicate that you wish the procedure to stop.

What are the possible disadvantages and risks of taking part?

Participants who meet any of the exclusion criteria should not take part in the study. This includes pregnant women or women who are breastfeeding, and women who plan to become pregnant. All women will therefore be asked to answer some short questions about their use of contraception to exclude the possibility of pregnancy.

What are the possible benefits of taking part?

Participants who complete the study will receive 10 course credits. Beyond this you should not expect to directly benefit from taking part in this research study. However, the information we get from this study may help us to understand more about anxiety disorders in the future.

What if new information becomes available?

We do not expect any information about the effects of the inhalation procedure to become available, but if this happens this information will be passed on to you immediately.

Will my taking part in this study be kept confidential?

All data is anonymized and confidential. You will be assigned a unique participant number that will be used to identify your data – your name will not be linked with or stored with any of your data. Any information and research study documentation taken for this research study will remain confidential and will be available only to the principal investigator and members of the research team directly involved in the project.

What will happen to the results of the research study?

When the study has been completed, data will be analyzed and the findings will be written up as part of an MSc dissertation thesis. You would not be identified in any way and if you would like a copy of the final paper, you may request this.

Who has reviewed the study?

The study has received approval from an appropriate ethics and Research Governance panels within the University of Southampton.

Who can I contact for further information?

For further queries, please contact my supervisor, Dr. Matt Garner:

Dr Matt Garner

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02380595926

02380718528

A.14 STUDY FOUR: DEBRIEF

EEG brain activity during 7.5% Carbon Dioxide Inhalation and breathing focus in healthy humans

Debriefing Statement (Version.1, 1/2/2013, Study ID: 5444)

Thank you for taking part in this experiment.

Study background: Inhalation of 7.5% CO₂ increases anxiety and autonomic arousal (e.g. heart rate, blood pressure, skin conductance) in humans and provides a novel, safe, experimental model of anxiety in healthy humans. Research using this model however, has not yet studied how certain patterns of brain activity respond to heightened feelings of anxiety and detection of threat induced by inhalation of CO₂.

The aim of this research was to investigate whether 7.5% CO₂ inhalation (in comparison to normal air inhalation) had any effect on patterns of brain activity measured using Electroencephalography (EEG). This study also examined the extent to which CO₂ inhalation effected physiological arousal, e.g. increase in heart rate, blood pressure and heightened self-report feelings of anxiety and mood.

Individuals also vary in their response to the inhalation of CO₂ with most individuals feeling completely normal by the end of the experimental session. Occasionally individuals report a mild headache for a short time afterwards.

In the unlikely event that you feel unwell please contact your General Practitioner or NHS Direct on 08454647.

If you are interested in finding out more about the rationale behind this research and the methods used in this project please see the references below:

1. Garner, M., Attwood, A., Baldwin, D. S., James, A., & Munafo, M. R. (2011). Inhalation of 7.5% Carbon Dioxide Increases Threat Processing in Humans. *Neuropsychopharmacology*, 36(8), 1557-1562. i
2. Bailey, J. E., Argyropoulos, S. V., Kendrick, A. H., & Nutt, D. J. (2005). Behavioral and cardiovascular effects of 7.5% CO₂ in human volunteers. *Depression and anxiety*, 21(1), p. 18-25.

During this study we have asked you to reflect on certain aspects of your physical and mental health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner. If you have more general worries during your time as a student in Southampton then please also be aware that Student Services or your personal tutor are available to provide support and advice.

Furthermore if you have any concerns or queries regarding any aspect of your participation in this study then please feel free to contact Dr Matt Garner on m.j.garner@soton.ac.uk or 02380595926 and he will be happy to discuss these with you.

A member of the research team will phone you tomorrow to check that you have not experienced any adverse events following your participation, and to discuss any additional queries that you might have.

Thank you again for your participation in this research.

Signature _____ Date _____

Name (print) _____

If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the Chair of the Ethics Committee, Psychology, University of Southampton, Southampton, SO17 1BJ. Phone: +44 (0)23 8059 4663, email slb1n10@soton.ac.uk

A.15 STUDY FOUR: ELECTRODE GROUPINGS

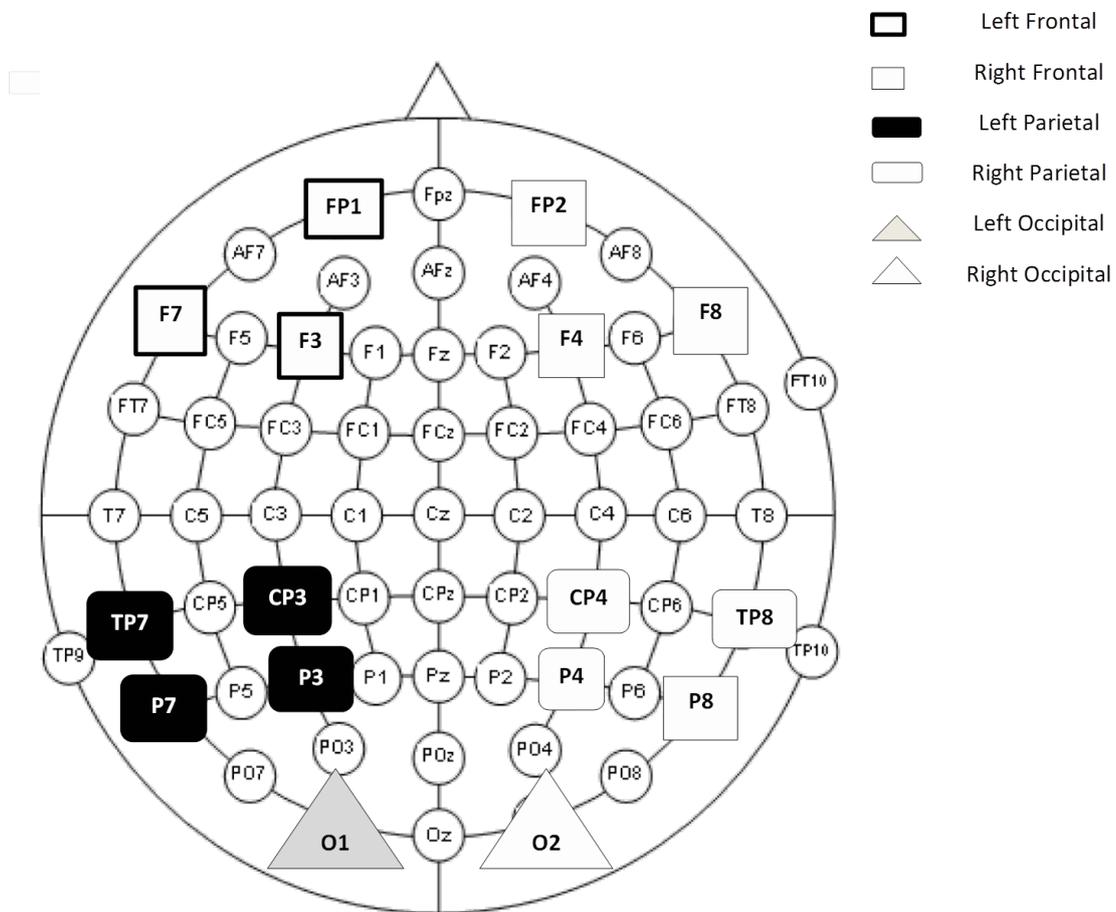


Figure 16 Study Four electrode groupings

A.16 STUDY FOUR: Group Characteristics Counterbalanced by Order Inhalation

There were no significant differences on measures of baseline autonomic arousal (heart rate, systolic and diastolic blood pressure) ($t(2,23)'s < -1.20, p's > .24$) or on the self-report anxiety, mood and attention measures (GAD7, STAI, ACS, PANAS positive affect, PANAS negative affect, VAS positive affect, VAS negative affect, VAS cognition, ASI and PSWQ) with ($t(2,23)'s < 2.01, p's > .06$) between those receiving CO₂ as first and as second.

Table 23

EEG Group Characteristics Counterbalanced by Order Inhalation

	Baseline				t(2,23) =
	CO ₂ first		Air first		
	M	SD	M	SD	
GAD-7	27.77	12.55	21.58	15.00	1.12, p=.27
STAI	35.54	6.85	33.00	6.68	0.92, p=.37
ACS	57.00	5.89	52.80	10.57	-0.95, p=.36
ASI	35.10	12.50	25.75	4.46	2.01, p=.06
PSWQ	34.00	9.93	43.20	11.12	1.83, p=.09
PANAS positive affect	34.69	7.45	32.58	4.48	0.85, p=.41

PANAS negative affect	14.31	4.44	13.22	3.44	0.63, p=.54
VAS negative affect	28.85	21.65	22.81	19.67	0.73, p=.48
VAS positive affect	106.38	20.56	107.54	18.51	-0.15, p=.88
VAS cognition	75.85	39.19	73.92	49.81	-0.11, p=.92
HR	74.69	10.63	74.33	9.87	0.09, p=.93
SBP	118.08	11.70	124.25	13.94	-1.20, p=.24
DBP	71.69	6.84	71.83	9.32	-0.04, p=.97

STAI= Spielberger Trait Anxiety Inventory; ACS = Attention Control Scale; ASI = Anxiety Sensitivity Index; PSWQ = Penn State Worry Questionnaire; Positive and Negative Affect Scale; HR= Heart rate; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure.

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